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Sir:

Transmitted herewith for filing is the patent application of

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For: HUMAN ANTIBODIES THAT BIND HUMAN IL-12 AND METHODS FOR PRODUCING

Enclosed are:

- ☒ This is a request for filing an application under 37 CFR 1.53(b).
- ☒ 144 pages of specification (including 14 pages of Appendix A), 30 pages of claims, 1 page of abstract.
- ☒ 14 sheets of drawings (Figures 1A-4).
- ☒ A Declaration, Petition and Power of Attorney (*unexecuted*).
- ☒ 145 pages of sequence listing (numbered 1-145).
- ☒ Transmittal Letter for Diskette of Sequence Listing.
- ☒ Diskette Containing Sequence Listing.

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INDEP. CLAIMS	41- 3	= 38	x 39	\$	OR	x 78	\$ 2964
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- ☒ Address all future communications (May only be completed by applicant, or attorney or agent of record) to Giulio A. DeConti, Jr., Esq. at **Customer Number: 000959** whose address is:

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HUMAN ANTIBODIES THAT BIND HUMAN IL-12 AND METHODS FOR PRODUCING

Related Applications

- 5 This application is a non-provisional application claiming priority to U.S. provisional application Serial No. 60/126,603, filed March 25, 1999, the contents of which are hereby incorporated by reference.

Background of the Invention

- 10 Human interleukin 12 (IL-12) has recently been characterized as a cytokine with a unique structure and pleiotropic effects (Kobayashi, *et al.* (1989) *J. Exp Med.* 170:827-845; Seder, *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:10188-10192; Ling, *et al.* (1995) *J. Exp Med.* 154:116-127; Podlaski, *et al.* (1992) *Arch. Biochem. Biophys.* 294:230-237). IL-12 plays a critical role in the pathology associated with several diseases involving
15 immune and inflammatory responses. A review of IL-12, its biological activities, and its role in disease can be found in Gately *et al.* (1998) *Ann. Rev. Immunol.* 16: 495-521.

- Structurally, IL-12 is a heterodimeric protein comprising a 35 kDa subunit (p35) and a 40 kDa subunit (p40) which are both linked together by a disulfide bridge (referred to as the "p70 subunit"). The heterodimeric protein is produced primarily by antigen-
20 presenting cells such as monocytes, macrophages and dendritic cells. These cell types also secrete an excess of the p40 subunit relative to p70 subunit. The p40 and p35 subunits are genetically unrelated and neither has been reported to possess biological activity, although the p40 homodimer may function as an IL-12 antagonist.

- Functionally, IL-12 plays a central role in regulating the balance between antigen
25 specific T helper type (Th1) and type 2 (Th2) lymphocytes. The Th1 and Th2 cells govern the initiation and progression of autoimmune disorders, and IL-12 is critical in the regulation of Th1-lymphocyte differentiation and maturation. Cytokines released by the Th1 cells are inflammatory and include interferon γ (IFN γ), IL-2 and lymphotoxin (LT). Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13 to facilitate humoral immunity,
30 allergic reactions, and immunosuppression.

 Consistent with the preponderance of Th1 responses in autoimmune diseases and the proinflammatory activities of IFN γ , IL-12 may play a major role in the pathology associated with many autoimmune and inflammatory diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), and Crohn's disease.

- 35 Human patients with MS have demonstrated an increase in IL-12 expression as documented by p40 mRNA levels in acute MS plaques. (Windhagen *et al.*, (1995) *J. Exp. Med.* 182: 1985-1996). In addition, *ex vivo* stimulation of antigen-presenting cells with CD40L-expressing T cells from MS patients resulted in increased IL-12 production

compared with control T cells, consistent with the observation that CD40/CD40L interactions are potent inducers of IL-12.

Elevated levels of IL-12 p70 have been detected in the synovia of RA patients compared with healthy controls (Morita et al (1998) *Arthritis and Rheumatism*. 41: 306-314). Cytokine messenger ribonucleic acid (mRNA) expression profile in the RA synovia identified predominantly Th1 cytokines. (Bucht et al., (1996) *Clin. Exp. Immunol.* 103: 347-367). IL-12 also appears to play a critical role in the pathology associated with Crohn's disease (CD). Increased expression of IFN γ and IL-12 has been observed in the intestinal mucosa of patients with this disease (Fais et al. (1994) *J. Interferon Res.* 14:235-238; Parronchi et al., (1997) *Am. J. Path.* 150:823-832; Monteleone et al., (1997) *Gastroenterology*. 112:1169-1178, and Berrebi et al., (1998) *Am. J. Path* 152:667-672). The cytokine secretion profile of T cells from the lamina propria of CD patients is characteristic of a predominantly Th1 response, including greatly elevated IFN γ levels (Fuss, et al., (1996) *J. Immunol.* 157:1261-1270). Moreover, colon tissue sections from CD patients show an abundance of IL-12 expressing macrophages and IFN γ expressing T cells (Parronchi et al (1997) *Am. J. Path.* 150:823-832).

Due to the role of human IL-12 in a variety of human disorders, therapeutic strategies have been designed to inhibit or counteract IL-12 activity. In particular, antibodies that bind to, and neutralize, IL-12 have been sought as a means to inhibit IL-12 activity. Some of the earliest antibodies were murine monoclonal antibodies (mAbs), secreted by hybridomas prepared from lymphocytes of mice immunized with IL-12 (see e.g., World Patent Application Publication No. WO 97/15327 by Strober et al.; Neurath et al. (1995) *J. Exp. Med.* 182:1281-1290; Duchmann et al. (1996) *J. Immunol.* 26:934-938). These murine IL-12 antibodies are limited for their use *in vivo* due to problems associated with administration of mouse antibodies to humans, such as short serum half life, an inability to trigger certain human effector functions and elicitation of an unwanted immune response against the mouse antibody in a human (the "human anti-mouse antibody" (HAMA) reaction).

In general, attempts to overcome the problems associated with use of fully-murine antibodies in humans, have involved genetically engineering the antibodies to be more "human-like." For example, chimeric antibodies, in which the variable regions of the antibody chains are murine-derived and the constant regions of the antibody chains are human-derived, have been prepared (Junghans, et al. (1990) *Cancer Res.* 50:1495-1502; Brown et al. (1991) *Proc. Natl. Acad. Sci.* 88:2663-2667; Kettleborough et al. (1991) *Protein Engineering*. 4:773-783). However, because these chimeric and humanized antibodies still retain some murine sequences, they still may elicit an

unwanted immune reaction, the human anti-chimeric antibody (HACA) reaction, especially when administered for prolonged periods.

A preferred IL-12 inhibitory agent to murine antibodies or derivatives thereof (e.g., chimeric or humanized antibodies) would be an entirely human anti-IL-12 antibody, since such an agent should not elicit the HAMA reaction, even if used for prolonged periods. However, such antibodies have not been described in the art and, therefore are still needed.

Summary of the Invention

10 The present invention provides human antibodies that bind human IL-12. The invention also relates to the treatment or prevention of acute or chronic diseases or conditions whose pathology involves IL-12, using the human anti-IL-12 antibodies of the invention.

In one aspect, the invention provides an isolated human antibody, or an antigen-binding portion thereof, that binds to human IL-12.

In one embodiment, the invention provides a selectively mutated human IL-12 antibody, comprising:

a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue such that it binds to human IL-12.

In a preferred embodiment, the invention provides a selectively mutated human IL-12 antibody, comprising:

a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis position with an activity enhancing amino acid residue such that it binds to human IL-12.

In another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof is selectively mutated at more than one preferred selective mutagenesis position, contact or hypermutation positions with an activity enhancing amino acid residue. In another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof is selectively mutated at no more than three preferred selective mutagenesis positions, contact or hypermutation positions. In another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof is selectively mutated at no more than two preferred selective mutagenesis position, contact or hypermutation positions. In yet another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof, is selectively mutated such that a target specificity affinity level is attained, the target level being improved over that attainable when selecting for an antibody against the same antigen using phage display technology. In

another preferred embodiment, the selectively mutated human IL-12 antibody further retains at least one desirable property or characteristic, *e.g.*, preservation of non-cross reactivity with other proteins or human tissues, preservation of epitope recognition, production of an antibody with a close to a germline immunoglobulin sequence.

5 In another embodiment, the invention provides an isolated human antibody, or antigen-binding portion thereof, that binds to human IL-12 and dissociates from human IL-12 with a K_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or
10 less. More preferably, the isolated human antibody or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-2} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-7} \text{ M}$ or less. More preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-3} \text{ s}^{-1}$ or
15 less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-8} \text{ M}$ or less. More preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-4} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-9} \text{ M}$ or less. More preferably, the isolated human antibody,
20 or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-5} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-10} \text{ M}$ or less. Even more preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-5} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast
25 proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-11} \text{ M}$ or less.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less;
- 30 b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2.

In a preferred embodiment, the isolated human antibody, or an antigen-binding
35 portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ

ID NO: 5; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6. In a preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and has a light chain variable region comprising the amino acid
5 sequence of SEQ ID NO: 8.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- 10 b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

In a preferred embodiment, the isolated human antibody, or an antigen-binding
15 portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID
20 NO: 14. In a preferred embodiment, the isolated human antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15; and has a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- 25 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID
30 NO: 18.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19; and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20. In a preferred embodiment, the isolated human antibody, or an antigen-binding
35 portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21; and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has the heavy chain variable region comprising the amino acid sequence

of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24. In a preferred embodiment, the isolated human antibody comprises a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions or any allelic variation thereof as discussed in Kabat *et al.* (Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), included herein by reference. In a more preferred embodiment, the antibody heavy chain constant region is IgG1. In another preferred embodiment, the isolated human antibody is a Fab fragment, or a F(ab')₂ fragment or a single chain Fv fragment.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹ M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 404-SEQ ID NO: 469; and
- c) has a light chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 534-SEQ ID NO: 579.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:335-SEQ ID NO: 403; and a light chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 506-SEQ ID NO: 533. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 288-SEQ ID NO: 334; and a light chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 470-SEQ ID NO: 505. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, comprising a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24. In a preferred embodiment, the isolated human antibody comprises a heavy chain constant region, or an Fab fragment or a F(ab')₂ fragment or a single chain Fv fragment as described above.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹ M or less;

- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

5 In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27; and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, which has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 32. In a preferred embodiment, the isolated human antibody comprises a heavy chain constant region, or an Fab fragment, or a F(ab')₂ fragment or a single chain Fv fragment as described above.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁶ M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and
- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- 5 b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold
10 higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and
- 15 c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein
20 said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- 25 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- 30 b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein
said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19, and a heavy chain
35 CDR1 comprising the amino acid sequence of SEQ ID NO: 21; and
- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:

- 22, or a mutant thereof having one or more amino acid substitutions at preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain
- 5 CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.

- The invention also provides nucleic acid molecules encoding antibodies, or antigen binding portions thereof, of the invention. A preferred isolated nucleic acid encodes the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17.
- 10 The isolated nucleic acid encoding an antibody heavy chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 19. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 21. In another
- 15 embodiment, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23. In another embodiment, the isolated nucleic acid encodes the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18. The isolated nucleic acid encoding an antibody light chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the
- 20 antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 20. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 22. In another embodiment, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.
- 25 In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which
- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
 - b) comprises a heavy chain CDR3 comprising the amino acid sequence of
- 30 SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a
- 35 heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

A preferred isolated nucleic acid encodes the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25. The isolated nucleic acid encoding an antibody heavy chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 27. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29. In another embodiment, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31. In another embodiment, the isolated nucleic acid encodes the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26. The isolated nucleic acid encoding an antibody light chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 28. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 30. In another embodiment, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 32.

In another aspect, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the $V_{\text{H}}3$ germline family, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

c) has a light chain variable region comprising an amino acid sequence selected from a member of the $V_{\lambda}1$ germline family, wherein the light chain variable

region has a mutation at a preferred selective mutagenesis position, contact position or hypermutation position with an activity enhancing amino acid residue.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- 5 a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

- b) has a heavy chain variable region comprising an amino acid sequence
10 selected from the group consisting of SEQ ID NOs: 595-667, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact position or hypermutation position with an activity enhancing amino acid residue.

- c) has a light chain variable region comprising an amino acid sequence
15 selected from the group consisting of SEQ ID NOs: 669-675, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- 20 a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

- b) has a heavy chain variable region comprising the COS-3 germline amino acid sequence, wherein the heavy chain variable region has a mutation at a
25 preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

- c) has a light chain variable region comprising the DPL8 germline amino acid sequence, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity
30 enhancing amino acid residue.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) that binds to human IL-12 and dissociates from human IL-12 with a
35 k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

- b) has a heavy chain variable region comprising an amino acid sequence

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selected from a member of the V_H3 germline family, wherein the heavy chain variable region comprises a CDR2 that is structurally similar to CDR2s from other V_H3 germline family members, and a CDR1 that is structurally similar to CDR1s from other V_H3 germline family members, and wherein the heavy chain variable region has a mutation at
5 a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue;

c) has a light chain variable region comprising an amino acid sequence selected from a member of the V_L1 germline family, wherein the light chain variable region comprises a CDR2 that is structurally similar to CDR2s from other V_L1 germline
10 family members, and a CDR1 that is structurally similar to CDR1s from other V_L1 germline family members, and wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

In a preferred embodiment, the isolated human antibody, or antigen binding
15 portion thereof, has a mutation in the heavy chain CDR3. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the light chain CDR3. In another embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the heavy chain CDR2. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof,
20 has a mutation in the light chain CDR2. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the heavy chain CDR1. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the light chain CDR1.

In another aspect, the invention provides recombinant expression vectors
25 carrying the antibody-encoding nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of making the antibodies of the invention by culturing the host cells of the invention.

In another aspect, the invention provides an isolated human antibody, or antigen-
30 binding portion thereof, that neutralizes the activity of human IL-12, and at least one additional primate IL-12 selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-12, cynomolgus IL-12 and rhesus IL-12, but which does not neutralize the activity of the mouse IL-12.

In another aspect, the invention provides a pharmaceutical composition
35 comprising the antibody or an antigen binding portion thereof, of the invention and a pharmaceutically acceptable carrier.

In another aspect, the invention provides a composition comprising the antibody or an antigen binding portion thereof, and an additional agent, for example, a therapeutic agent.

In another aspect, the invention provides a method for inhibiting human IL-12 activity comprising contacting human IL-12 with the antibody of the invention, *e.g.*, J695, such that human IL-12 activity is inhibited.

In another aspect, the invention provides a method for inhibiting human IL-12 activity in a human subject suffering from a disorder in which IL-12 activity is detrimental, comprising administering to the human subject the antibody of the invention, *e.g.*, J695, such that human IL-12 activity in the human subject is inhibited. The disorder can be, for example, Crohn's disease, multiple sclerosis or rheumatoid arthritis.

In another aspect, the invention features a method for improving the activity of an antibody, or an antigen binding portion thereof, to attain a predetermined target activity, comprising:

- a) providing a parent antibody a antigen-binding portion thereof;
- b) selecting a preferred selective mutagenesis position selected from group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.
- c) individually mutating the selected preferred selective mutagenesis position to at least two other amino acid residues to hereby create a first panel of mutated antibodies, or antigen binding portions thereof;
- d) evaluating the activity of the first panel of mutated antibodies, or antigen binding portions thereof to determined if mutation of a single selective mutagenesis position produces an antibody or antigen binding portion thereof with the predetermined target activity or a partial target activity;
- e) combining in a stepwise fashion, in the parent antibody, or antigen binding portion thereof, individual mutations shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof.
- f) evaluating the activity of the combination antibodies, or antigen binding portions thereof to determined if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity.
- g) if steps d) or f) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result an antibody with only a partial activity, additional amino acid residues selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 are mutated to at least two other amino acid residues to thereby create a second panel of mutated antibodies or antigen-binding portions thereof;

h) evaluating the activity of the second panel of mutated antibodies or antigen binding portions thereof, to determine if mutation of a single amino acid residue selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 results an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

i) combining in stepwise fashion in the parent antibody, or antigen-binding portion thereof, individual mutations of step g) shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof;

j) evaluating the activity of the combination antibodies or antigen binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity;

k) if steps h) or j) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result in an antibody with only a partial activity, additional amino acid residues selected from the group consisting of H33B, H52B and L31A are mutated to at least two other amino acid residues to thereby create a third panel of mutated antibodies or antigen binding portions thereof;

l) evaluating the activity of the third panel of mutated antibodies or antigen binding portions thereof, to determine if a mutation of a single amino acid residue selected from the group consisting of H33B, H52B and L31A resulted in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

m) combining in a stepwise fashion in the parent antibody, or antigen binding portion thereof, individual mutation of step k) shown to have an improved activity, to form combination antibodies, or antigen binding portions, thereof;

n) evaluating the activity of the combination antibodies or antigen-binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity to thereby produce an antibody or antigen binding portion thereof with a predetermined target activity.

In another aspect, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;

e) repeating steps b) through d) for at least one other contact or hypermutation position;

5 f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;
10 until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In one embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof;
15 that was obtained by selection in a phage-display system but whose activity is not further improved by mutagenesis in said phage-display system;

b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;

20 c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding
25 portions thereof, relative to the parent antibody or antigen-binding portion thereof;

e) repeating steps b) through d) for at least one other contact or hypermutation position;

f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies,
30 or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;
until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

35 In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. In another preferred embodiment, the hypermutation positions are selected

from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.

- 5 In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof ;
- 10 b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby
- 15 create a panel of mutated antibodies, or antigen-binding portions thereof and expressing said panel in an appropriate expression system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- 20 e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristics, wherein the property or characteristic is one that needs to be retained in the antibody;
- until an antibody, or antigen-binding portion thereof, with an improved activity and at
- 25 least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

- In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93,
- 30 L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred
- 35 embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope

preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, *i.e.* recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, *i.e.* recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment of the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) repeating steps a) through e) for at least one other preferred selective mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer

preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- 5 a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or
- 10 hypermutation position;
- c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- 15 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one
- 20 other property or characteristics, wherein the property or characteristic is one that needs to be retained;
- until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.
- 25 In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
- 30 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other
- 35 characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline

immunoglobulin sequence. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.
- f) repeating steps a) through e) for at least one other preferred selective mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained other characteristic, to form combination antibodies, or antigen-binding portions thereof; and

- 5 h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

- 10 In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
15 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other
20 characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective
25 mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
30 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e.
35 recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
 - b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
 - c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
 - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
 - e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic;
- until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;
- e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33,

H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and;

c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other position within the CDR which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 ;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and other property or characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies or antigen-binding portions thereof, relative to the parent antibody or antigen-portion thereof, for changes in at least one other property or characteristic;

10 f) repeating steps b) through e) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity and the retention of at least one other characteristic or property of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In another embodiment the invention provides a method to improve the affinity
25 of an antibody or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other characteristic or property until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding
5 portion thereof, is obtained.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;
b) selecting an amino acid residue within a complementarity determining region
10 (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions
15 thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies or antigen-binding portions thereof,
20 relative to the parent antibody or antigen-portion thereof, for changes in at least one other property or characteristic;

f) repeating steps b) through e) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32,
25 L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity but not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof with at least one retained property or
30 characteristic; and

h) evaluating the activity and the retention of at least one property of characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an
35 improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, without affecting other characteristics, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity and retained other characteristic or property, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;
- d) evaluating the activity and retention of at least one other characteristic or property of the panel of mutated antibodies, or antigen-binding portions thereof, relative

to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b nor other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and not to affect at least one other characteristic or property, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and retention of at least one other characteristic or property of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one other retained characteristic or property, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

Brief Description of the Drawings

Figures 1A-1B show the heavy chain variable region amino acid sequence alignments of a series of human antibodies that bind human IL-12 compared to germline sequences Cos-3/JH3 and Dpl18 Lv1042. Kabat numbering is used to identify amino acid positions. For the Joe 9 wild type, the full sequence is shown. For the other antibodies, only those amino acids positions that differ from Joe 9 wild type are shown.

Figures 1C-1D show the light chain variable region amino acid sequence alignments of a series of human antibodies that bind human IL-12. Kabat numbering is used to identify amino acid positions. For the Joe 9 wild type, the full sequence is shown. For the other antibodies, only those amino acids positions that differ from Joe 9 wild type are shown.

Figures 2A-2E show the CDR positions in the heavy chain of the Y61 antibody that were mutated by site-directed mutagenesis and the respective amino acid substitutions at each position. The graphs at the right of the figures show the off-rates for the substituted antibodies (black bars) as compared to unmutated Y61 (open bar).

Figures 2F-2H show the CDR positions in the light chain of the Y61 antibody that were mutated by site-directed mutagenesis and the respective amino acid substitutions at each position. The graphs at the right of the figures show the off-rates for the substituted antibodies (black bars) as compared to unmutated Y61 (open bar).

5 Figure 3 demonstrates the *in vivo* efficacy of the human anti-IL-12 antibody J695, on plasma neopterin levels in cynomolgus monkeys.

Figure 4 shows a graph of mean arthritic score versus days after immunization of mice with collagen, demonstrating that treatment with C17.15 significantly decreases arthritis-related symptoms as compared to treatment with rat IgG.

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Detailed Description of the Invention

In order that the present invention may be more readily understood, certain terms are first defined.

15 The term "activity enhancing amino acid residue" includes an amino acid residue which improves the activity of the antibody. It should be understood that the activity enhancing amino acid residue may replace an amino acid residue at a contact, hypermutation or preferred selective mutagenesis position and, further, more than one activity enhancing amino acid residue can be present within one or more CDRs. An activity enhancing amino acid residue include, an amino acid residue that improves the
20 binding specificity/affinity of an antibody, for example anti-human IL-12 antibody binding to human IL-12. The activity enhancing amino acid residue is also intended to include an amino acid residue that improves the neutralization potency of an antibody, for example, the human IL-12 antibody which inhibits human IL-12.

25 The term "antibody" includes an immunoglobulin molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL)
30 and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to
35 carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The term "antigen-binding portion" of an antibody (or "antibody portion") includes fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, hIL-12). It has been shown that the antigen-binding function of an antibody can be

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separately with the germline sequences in the VBASE database to identify the sequences with the highest homology. Differences in the human antibody of the invention are returned to the germline sequence by mutating defined nucleotide positions encoding such different amino acid. The role of each amino acid thus identified as candidate for backmutation should be investigated for a direct or indirect role in antigen binding and any amino acid found after mutation to affect any desirable characteristic of the human antibody should not be included in the final human antibody; as an example, activity enhancing amino acids identified by the selective mutagenesis approach will not be subject to backmutation. To minimize the number of amino acids subject to backmutation those amino acid positions found to be different from the closest germline sequence but identical to the corresponding amino acid in a second germline sequence can remain, provided that the second germline sequence is identical and colinear to the sequence of the human antibody of the invention for at least 10, preferably 12 amino acids, on both sides of the amino acid in question. Backmutation may occur at any stage of antibody optimization; preferably, backmutation occurs directly before or after the selective mutagenesis approach. More preferably, backmutation occurs directly before the selective mutagenesis approach.

The phrase "human interleukin 12" (abbreviated herein as hIL-12, or IL-12), as used herein, includes a human cytokine that is secreted primarily by macrophages and dendritic cells. The term includes a heterodimeric protein comprising a 35 kD subunit (p35) and a 40 kD subunit (p40) which are both linked together with a disulfide bridge. The heterodimeric protein is referred to as a "p70 subunit". The structure of human IL-12 is described further in, for example, Kobayashi, *et al.* (1989) *J. Exp Med.* 170:827-845; Seder, *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:10188-10192; Ling, *et al.* (1995) *J. Exp Med.* 154:116-127; Podlaski, *et al.* (1992) *Arch. Biochem. Biophys.* 294:230-237. The term human IL-12 is intended to include recombinant human IL-12 (rh IL-12), which can be prepared by standard recombinant expression methods.

The terms "Kabat numbering", "Kabat definitions" and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.* hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat *et al.* (1971) *Ann. NY Acad. Sci.* 190:382-391 and , Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable

region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

The Kabat numbering is used herein to indicate the positions of amino acid modifications made in antibodies of the invention. For example, the Y61 anti-IL-12 antibody can be mutated from serine (S) to glutamic acid (E) at position 31 of the heavy chain CDR1 (H31S → E), or glycine (G) can be mutated to tyrosine (Y) at position 94 of the light chain CDR3 (L94G → Y).

The term "human antibody" includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences as described by Kabat *et al.* (See Kabat, *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. The mutations preferably are introduced using the "selective mutagenesis approach" described herein. The human antibody can have at least one position replaced with an amino acid residue, *e.g.*, an activity enhancing amino acid residue which is not encoded by the human germline immunoglobulin sequence. The human antibody can have up to twenty positions replaced with amino acid residues which are not part of the human germline immunoglobulin sequence. In other embodiments, up to ten, up to five, up to three or up to two positions are replaced. In a preferred embodiment, these replacements are within the CDR regions as described in detail below. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The phrase "recombinant human antibody" includes human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further in Section II, below), antibodies isolated from a recombinant, combinatorial human antibody library (described further in Section III, below), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor, L.D., *et al.* (1992) *Nucl. Acids Res.* 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences (See Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services,

NIH Publication No. 91-3242). In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. In certain embodiments, however, such recombinant antibodies are the result of selective mutagenesis approach or backmutation or both.

An "isolated antibody" includes an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds hIL-12 is substantially free of antibodies that specifically bind antigens other than hIL-12). An isolated antibody that specifically binds hIL-12 may bind IL-12 molecules from other species (discussed in further detail below). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

A "neutralizing antibody" (or an "antibody that neutralized hIL-12 activity") includes an antibody whose binding to hIL-12 results in inhibition of the biological activity of hIL-12. This inhibition of the biological activity of hIL-12 can be assessed by measuring one or more indicators of hIL-12 biological activity, such as inhibition of human phytohemagglutinin blast proliferation in a phytohemagglutinin blast proliferation assay (PHA), or inhibition of receptor binding in a human IL-12 receptor binding assay (see Example 3-Interferon-gamma Induction Assay). These indicators of hIL-12 biological activity can be assessed by one or more of several standard *in vitro* or *in vivo* assays known in the art (see Example 3).

The term "activity" includes activities such as the binding specificity/affinity of an antibody for an antigen, for example, an anti-hIL-12 antibody that binds to an IL-12 antigen and/or the neutralizing potency of an antibody, for example, an anti-hIL-12 antibody whose binding to hIL-12 inhibits the biological activity of hIL-12, *e.g.* inhibition of PHA blast proliferation or inhibition of receptor binding in a human IL-12 receptor binding assay (see Example 3).

The phrase "surface plasmon resonance" includes an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Example 5 and Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnsson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277.

The term " K_{off} ", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

The term " K_d ", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction.

5 The phrase "nucleic acid molecule" includes DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

10 The phrase "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody portions (*e.g.*, VH, VL, CDR3) that bind hIL-12 including "isolated antibodies"), includes a nucleic acid molecule in which the nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than hIL-12, which other sequences may naturally flank the nucleic acid in human genomic DNA. Thus, for example, an isolated nucleic acid of the invention encoding a
15 VH region of an anti-IL-12 antibody contains no other sequences encoding other VH regions that bind antigens other than IL-12. The phrase "isolated nucleic acid molecule" is also intended to include sequences encoding bivalent, bispecific antibodies, such as diabodies in which VH and VL regions contain no other sequences other than the sequences of the diabody.

20 The term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of
25 autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes
30 to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However,
35 the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The phrase "recombinant host cell" (or simply "host cell") includes a cell into which a recombinant expression vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding
5 generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

The term "modifying", as used herein, is intended to refer to changing one or more amino acids in the antibodies or antigen-binding portions thereof. The change can
10 be produced by adding, substituting or deleting an amino acid at one or more positions. The change can be produced using known techniques, such as PCR mutagenesis.

The phrase "contact position" includes an amino acid position of in the CDR1, CDR2 or CDR3 of the heavy chain variable region or the light chain variable region of an antibody which is occupied by an amino acid that contacts antigen in one of the
15 twenty-six known antibody-antigen structures. If a CDR amino acid in any of the 26 known solved structures of antibody-antigen complexes contacts the antigen, then that amino acid can be considered to occupy a contact position. Contact positions have a higher probability of being occupied by an amino acid which contact antigen than non-contact positions. Preferably a contact position is a CDR position which contains an
20 amino acid that contacts antigen in greater than 3 of the 26 structures (>11.5 %). Most preferably a contact position is a CDR position which contains an amino acid that contacts antigen in greater than 8 of the 25 structures (>32%).

The term "hypermutation position" includes an amino acid residue that occupies position in the CDR1, CDR2 or CDR3 region of the heavy chain variable region or the
25 light chain variable region of an antibody that is considered to have a high frequency or probability for somatic hypermutation during *in vivo* affinity maturation of the antibody. "High frequency or probability for somatic hypermutation" includes frequencies or probabilities of a 5 to about 40% chance that the residue will undergo somatic hypermutation during *in vivo* affinity maturation of the antibody. It should be
30 understood that all ranges within this stated range are also intended to be part of this invention, e.g., 5 to about 30%, e.g., 5 to about 15%, e.g., 15 to about 30%.

The term "preferred selective mutagenesis position" includes an amino acid residue that occupies a position in the CDR1, CDR2 or CDR3 region of the heavy chain variable region or the light chain variable region which can be considered to be both a
35 contact and a hypermutation position.

The phrase "selective mutagenesis approach" includes a method of improving the activity of an antibody by selecting and individually mutating CDR amino acids at at least one preferred selective mutagenesis position, hypermutation, and/or contact

position. A "selectively mutated" human antibody is an antibody which contains a mutation at a position selected using a selective mutagenesis approach. In another embodiment, the selective mutagenesis approach is intended to provide a method of preferentially mutating selected individual amino acid residues in the CDR1, CDR2 or CDR3 of the heavy chain variable region (hereinafter H1, H2, and H3, respectively), or the CDR1, CDR2 or CDR3 of the light chain variable region (hereinafter referred to as L1, L2, and L3, respectively) of an antibody. Amino acid residues may be selected from preferred selective mutagenesis positions, contact positions, or hypermutation positions. Individual amino acids are selected based on their position in the light or heavy chain variable region. It should be understood that a hypermutation position can also be a contact position. In an embodiment, the selective mutagenesis approach is a "targeted approach". The language "targeted approach" is intended to include a method of preferentially mutating selected individual amino acid residues in the CDR1, CDR2 or CDR3 of the heavy chain variable region or the CDR1, CDR2 or CDR3 of the light chain variable region of an antibody in a targeted manner, *e.g.*, a "Group-wise targeted approach" or "CDR-wise targeted approach". In the "Group-wise targeted approach", individual amino acid residues in particular groups are targeted for selective mutations including groups I (including L3 and H3), II (including H2 and L1) and III (including L2 and H1), the groups being listed in order of preference for targeting. In the "CDR-wise targeted approach", individual amino acid residues in particular CDRs are targeted for selective mutations with the order of preference for targeting as follows: H3, L3, H2, L1, H1 and L2. The selected amino acid residue is mutated, *e.g.*, to at least two other amino acid residues, and the effect of the mutation on the activity of the antibody is determined. Activity is measured as a change in the binding specificity/affinity of the antibody, and/or neutralization potency of the antibody. It should be understood that the selective mutagenesis approach can be used for the optimization of any antibody derived from any source including phage display, transgenic animals with human IgG germline genes, human antibodies isolated from human B-cells. Preferably, the selective mutagenesis approach is used on antibodies which can not be optimized further using phage display technology. It should be understood that antibodies from any source including phage display, transgenic animals with human IgG germline genes, human antibodies isolated from human B-cells can be subject to backmutation prior to or after the selective mutagenesis approach.

The term "activity enhancing amino acid residue" includes an amino acid residue which improves the activity of the antibody. It should be understood that the activity enhancing amino acid residue may replace an amino acid residue at a preferred selective mutagenesis position, contact position, or a hypermutation position and, further, more than one activity enhancing amino acid residue can be present within one or more CDRs.

An activity enhancing amino acid residue include, an amino acid residue that improves the binding specificity/affinity of an antibody, for example anti-human IL-12 antibody binding to human IL-12. The activity enhancing amino acid residue is also intended to include an amino acid residue that improves the neutralization potency of an antibody,
 5 for example, the human IL-12 antibody which inhibits human IL-12.

Various aspects of the invention are described in further detail in the following subsections.

I. Human Antibodies that Bind Human IL-12

10 This invention provides isolated human antibodies, or antigen-binding portions thereof, that bind to human IL-12. Preferably, the human antibodies of the invention are recombinant, neutralizing human anti-hIL-12 antibodies. Antibodies of the invention that bind to human IL-12 can be selected, for example, by screening one or more human V_L and V_H cDNA libraries with hIL-12, such as by phage display techniques as
 15 described in Example 1. Screening of human V_L and V_H cDNA libraries initially identified a series of anti-IL-12 antibodies of which one antibody, referred to herein as "Joe 9" (or "Joe 9 wild type"), was selected for further development. Joe 9 is a relatively low affinity human IL-12 antibody (*e.g.*, a K_{off} of about 0.1 sec^{-1}), yet is useful for specifically binding and detecting hIL-12. The affinity of the Joe 9 antibody was
 20 improved by conducting mutagenesis of the heavy and light chain CDRs, producing a panel of light and heavy chain variable regions that were "mixed and matched" and further mutated, leading to numerous additional anti-hIL-12 antibodies with increased affinity for hIL-12 (see Example 1, Table 2 (see Appendix A) and the sequence alignments of Figures 1A-D).

25 Of these antibodies, the human anti-hIL-12 antibody referred to herein as Y61 demonstrated a significant improvement in binding affinity (*e.g.*, a K_{off} of about $2 \times 10^{-4} \text{ sec}^{-1}$). The Y61 anti-hIL-12 antibody was selected for further affinity maturation by individually mutating specific amino acids residues within the heavy and light chain CDRs. Amino acids residues of Y61 were selected for site-specific mutation (selective
 30 mutagenesis approach) based on the amino acid residue occupying a preferred selective mutagenesis position, contact and/or a hypermutation position. A summary of the substitutions at selected positions in the heavy and light chain CDRs is shown in Figures 2A-2H. A preferred recombinant neutralizing antibody of the invention, referred to herein as J695, resulted from a Gly to Tyr substitution at position 50 of the
 35 light chain CDR2 of Y61, and a Gly to Tyr substitution at position 94 of the light chain CDR3 of Y61.

Amino acid sequence alignments of the heavy and light chain variable regions of a panel of anti-IL-12 antibodies of the invention, on the lineage from Joe 9 wild type to

J695, are shown in Figures 1A-1D. These sequence alignments allowed for the identification of consensus sequences for preferred heavy and light chain variable regions of antibodies of the invention that bind hIL-12, as well as consensus sequences for the CDR3, CDR2, and CDR1, on the lineage from Joe 9 to J695. Moreover, the

5 Y61 mutagenesis analysis summarized in Figures 2A-2H allowed for the identification of consensus sequences for heavy and light chain variable regions that bind hIL-12, as well as consensus sequences for the CDR3, CDR2, and CDR1 that bind hIL-12 on the lineage from Y61 to J695 that encompasses sequences with modifications from Y61 yet that retain good hIL-12 binding characteristics. Preferred CDR, VH and VL sequences

10 of the invention (including consensus sequences) as identified by sequence identifiers in the attached Sequence Listing, are summarized below.

SEQ ID NO:	ANTIBODY CHAIN	REGION	SEQUENCE
1	Consensus Joe 9 to J695	CDR H3	(H/S) -G-S- (H/Y) -D- (N/T/Y)
2	Consensus Joe 9 to J695	CDR L3	Q- (S/T) -Y- (D/E) - (S/R/K) - (S/G/Y) - (L/F/T/S) - (R/S/T/W/H) - (G/P) - (S/T/A/L) - (R/S/M/T/L) - (V/I/T/M/L)
3	Consensus Joe 9 to J695	CDR H2	F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G
4	Consensus Joe 9 to J695	CDR L2	(G/Y) -N- (D/S) - (Q/N) -R-P-S
5	Consensus Joe 9 to J695	CDR H1	F-T-F-S- (S/E) -Y-G-M-H
6	Consensus Joe 9 to J695	CDR L1	(S/T) -G- (G/S) - (R/S) -S-N-I- (G/V) - (S/A) - (N/G/Y) - (T/D) -V- (K/H)
7	Consensus Joe 9 to J695	VH	(full VH sequence; see sequence listing)
8	Consensus Joe 9 to J695	VL	(full VL sequence; see sequence listing)
9	Consensus Y61 to J695	CDR H3	H- (G/V/C/H) - (S/T) - (H/T/V/R/I) - (D/S) - (N/K/A/T/S/F/W/H)
10	Consensus Y61 to J695	CDR L3	Q-S-Y- (D/S) - (Xaa) - (G/D/Q/L/F/R/H/N/Y) -T-H-P-A-L-L
11	Consensus Y61 to J695	CDR H2	(F/T/Y) -I- (R/A) -Y- (D/S/E/A) - (G/R) -S- (Xaa) -K- (Y/E) -Y-A-D-S-V-K-G
12	Consensus Y61 to J695	CDR L2	(G/Y/S/T/N/Q) -N-D-Q-R-P-S
13	Consensus Y61 to J695	CDR H1	F-T-F- (Xaa) - (Xaa) - (Y/H) - (G/M/A/N/S) -M-H
14	Consensus Y61 to J695	CDR L1	S-G-G-R-S-N-I-G- (S/C/R/N/D/T) - (N/M/I) - (T/Y/D/H/K/P) -V-K
15	Consensus Y61 to J695	VH	(full VH sequence; see sequence listing)
16	Consensus Y61 to J695	VL	(full VL sequence; see sequence listing)
17	Y61	CDR H3	H-G-S-H-D-N

18	Y61	CDR L3	Q-S-Y-D-R-G-T-H-P-A-L-L
19	Y61	CDR H2	F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G
20	Y61	CDR L2	G-N-D-Q-R-P-S
21	Y61	CDR H1	F-T-F-S-S-Y-G-M-H
22	Y61	CDR L1	S-G-G-R-S-N-I-G-S-N-T-V-K
23	Y61	VH	(full VH sequence; see sequence listing)
24	Y61	VL	(full VL sequence; see sequence listing)
25	J695	CDR H3	H-G-S-H-D-N
26	J695	CDR L3	Q-S-Y-D-R-Y-T-H-P-A-L-L
27	J695	CDR H2	F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G
28	J695	CDR L2	Y-N-D-Q-R-P-S
29	J695	CDR H1	F-T-F-S-S-Y-G-M-H
30	J695	CDR L1	S-G-S-R-S-N-I-G-S-N-T-V-K
31	J695	VH	(full VH sequence; see sequence listing)
32	J695	VL	(full VL sequence; see sequence listing)

Antibodies produced from affinity maturation of Joe 9 wild type were functionally characterized by surface plasmon resonance analysis to determine the K_d and K_{off} rate. A series of antibodies were produced having a K_{off} rate within the range of about 0.1 s^{-1} to about $1 \times 10^{-5} \text{ s}^{-1}$, and more preferably a K_{off} of about $1 \times 10^{-4} \text{ s}^{-1}$ to $1 \times 10^{-5} \text{ s}^{-1}$ or less. Antibodies were also characterized *in vitro* for their ability to inhibit phytohemagglutinin (PHA) blast proliferation, as described in Example 3. A series of antibodies were produced having an IC_{50} value in the range of about $1 \times 10^{-6} \text{ M}$ to about $1 \times 10^{-11} \text{ M}$, more preferably about $1 \times 10^{-10} \text{ M}$ to $1 \times 10^{-11} \text{ M}$ or less.

Accordingly, in one aspect, the invention provides an isolated human antibody, or antigen-binding portion thereof, that binds to human IL-12 and dissociates from human IL-12 with a K_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less. In preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-2} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-7} \text{ M}$ or less. In more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-3} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-8} \text{ M}$ or less. In more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-4} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-9} \text{ M}$ or less. In more preferred embodiments, the isolated human IL-12

antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-5} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-10} \text{ M}$ or less. In even more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding

5 portion thereof, dissociates from human IL-12 with a K_{off} rate constant of $1 \times 10^{-5} \text{ s}^{-1}$ or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-11} \text{ M}$ or less.

The dissociation rate constant (K_{off}) of an IL-12 antibody can be determined by surface plasmon resonance (see Example 5). Generally, surface plasmon resonance

10 analysis measures real-time binding interactions between ligand (recombinant human IL-12 immobilized on a biosensor matrix) and analyte (antibodies in solution) by surface plasmon resonance (SPR) using the BIAcore system (Pharmacia Biosensor, Piscataway, NJ). Surface plasmon analysis can also be performed by immobilizing the analyte

15 (antibodies on a biosensor matrix) and presenting the ligand (recombinant IL-12 in solution). Neutralization activity of IL-12 antibodies, or antigen binding portions thereof, can be assessed using one or more of several suitable *in vitro* assays (see Example 3).

It is well known in the art that antibody heavy and light chain CDRs play an important role in the binding specificity/affinity of an antibody for an antigen.

20 Accordingly, the invention encompasses human antibodies having light and heavy chain CDRs of Joe 9, as well as other antibodies having CDRs that have been modified to improve the binding specificity/affinity of the antibody. As demonstrated in Example 1, a series of modifications to the light and heavy chain CDRs results in affinity maturation of human anti-hIL-12 antibodies. The heavy and light chain variable region

25 amino acid sequence alignments of a series of human antibodies ranging from Joe 9 wild type to J695 that bind human IL-12 is shown in Figures 1A-1D. Consensus sequence motifs for the CDRs of antibodies can be determined from the sequence alignment (as summarized in the table above). For example, a consensus motif for the VH CDR3 of the lineage from Joe 9 to J695 comprises the amino acid sequence: (H/S)-G-S-(H/Y)-D-

30 (N/T/Y) (SEQ ID NO: 1), which encompasses amino acids from position 95 to 102 of the consensus HCVR shown in SEQ ID NO: 7. A consensus motif for the VL CDR3 comprises the amino acid sequence: Q-(S/T)-Y-(D/E)-(S/R/K)-(S/G/Y)-(L/F/T/S)-(R/S/T/W/H)-(G/P)-(S/T/A/L)-(R/S/M/T/L-V/I/T/M/L) (SEQ ID NO: 2), which encompasses amino acids from position 89 to 97 of the consensus LCVR shown in SEQ

35 ID NO: 8.

Accordingly, in another aspect, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-6} M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1; and
- 5 c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2.

In a preferred embodiment, the antibody further comprises a VH CDR2 comprising the amino acid sequence: F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G (SEQ ID NO: 3) (which encompasses amino acids from position 50 to 65 of the consensus

10 HCVR comprising the amino acid sequence SEQ ID NO: 7) and further comprises a VL CDR2 comprising the amino acid sequence: (G/Y)-N-(D/S)-(Q/N)-R-P-S (SEQ ID NO: 4) (which encompasses amino acids from position 50 to 56 of the consensus LCVR comprising the amino acid sequence SEQ ID NO: 8).

In another preferred embodiment, the antibody further comprises a VH CDR1

15 comprising the amino acid sequence: F-T-F-S-(S/E)-Y-G-M-H (SEQ ID NO: 5) (which encompasses amino acids from position 27 to 35 of the consensus HCVR comprising the amino acid sequence SEQ ID NO: 7) and further comprises a VL CDR1 comprising the amino acid sequence: (S/T)-G-(G/S)-(R/S)-S-N-I-(G/V)-(S/A)-(N/G/Y)-(T/D)-V-(K/H) (SEQ ID NO: 6) (which encompasses amino acids from position 24 to 34 of the

20 consensus LCVR comprising the amino acid sequence SEQ ID NO: 8).

In yet another preferred embodiment, the antibody of the invention comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 7 and a LCVR comprising the amino acid sequence of SEQ ID NO: 8.

Additional consensus motifs can be determined based on the mutational analysis

25 performed on Y61 that led to the J695 antibody (summarized in Figures 2A-2H). As demonstrated by the graphs shown in Figures 2A-2H, certain residues of the heavy and light chain CDRs of Y61 were amenable to substitution without significantly impairing the hIL-12 binding properties of the antibody. For example, individual substitutions at position 30 in CDR H1 with twelve different amino acid residues did not significantly

30 reduce the K_{off} rate of the antibody, indicating that is position is amenable to substitution with a variety of different amino acid residues. Thus, based on the mutational analysis (i.e., positions within Y61 that were amenable to substitution by other amino acid residues) consensus motifs were determined. The consensus motifs for the heavy and light chain CDR3s are shown in SEQ ID NOs: 9 and 10, respectively,

35 consensus motifs for the heavy and light chain CDR2s are shown in SEQ ID NOs: 11 and 12, respectively, and consensus motifs for the heavy and light chain CDR1s are shown in SEQ ID NOs: 13 and 14, respectively. Consensus motifs for the VH and VL regions are shown in SEQ ID NOs: 15 and 16, respectively.

Accordingly, in one aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- 5 b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

In a preferred embodiment, the antibody further comprises a VH CDR2
10 comprising the amino acid sequence of SEQ ID NO: 11 and further comprises a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 12.

In another preferred embodiment, the antibody further comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 13 and further comprises a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

15 In yet another preferred embodiment, the antibody of the invention comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 15 and a LCVR comprising the amino acid sequence of SEQ ID NO: 16.

A preferred antibody of the invention, the human anti-hIL-12 antibody Y61, was produced by affinity maturation of Joe 9 wild type by PCR mutagenesis of the CDR3 (as
20 described in Example 1). Y61 had an improved specificity/binding affinity determined by surface plasmon resonance and by *in vitro* neutralization assays. The heavy and light chain CDR3s of Y61 are shown in SEQ ID NOs: 17 and 18, respectively, the heavy and light chain CDR2s of Y61 are shown in SEQ ID NOs: 19 and 20, respectively, and the heavy and light chain CDR1s of Y61 are shown in SEQ ID NOs: 21 and 22,
25 respectively. The VH of Y61 has the amino acid sequence of SEQ ID NO: 23 and the VL of Y61 has the amino acid sequence of SEQ ID NO: 24 (these sequences are also shown in Figures 1A-1D, aligned with Joe9).

Accordingly, in another aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- 30 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID
35 NO: 18.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ

ID NO: 19 and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20.

In another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof has a heavy chain CDR1 comprising the amino acid sequence of
5 SEQ ID NO: 21 and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.

In yet another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, comprising a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising
10 the amino acid sequence of SEQ ID NO: 24.

In certain embodiments, the full length antibody comprises a heavy chain constant region, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions, and any allotypic variant therein as described in Kabat (, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of
15 Health and Human Services, NIH Publication No. 91-3242). Preferably, the antibody heavy chain constant region is an IgG1 heavy chain constant region. Alternatively, the antibody portion can be an Fab fragment, an F(ab')₂ fragment or a single chain Fv fragment.

Modifications of individual residues of Y61 led to the production of a panel of
20 antibodies shown in Figures 2A-2H. The specificity/binding affinity of each antibody was determined by surface plasmon resonance and/or by *in vitro* neutralization assays.

Accordingly, in another aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay
25 with an IC₅₀ of 1 x 10⁻⁹ M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 404-SEQ ID NO: 469; and
- c) has a light chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 534-SEQ ID NO: 579.

In preferred embodiment, the isolated human antibody, or an antigen-binding
30 portion thereof, has a heavy chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:335-SEQ ID NO: 403; and a light chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 506-SEQ ID NO: 533.

In another preferred embodiment, the isolated human antibody, or an antigen-binding
35 portion thereof, has a heavy chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 288-SEQ ID NO: 334; and a light

chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 470-SEQ ID NO: 505.

In yet another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, comprising a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

In certain embodiments, the full length antibody comprising a heavy chain constant region such as IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions and any allotypic variant therein as described in Kabat (, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Preferably, the antibody heavy chain constant region is an IgG1 heavy chain constant region. Alternatively, the antibody portion can be a Fab fragment, an F(ab')₂ fragment or a single chain Fv fragment.

A particularly preferred recombinant, neutralizing antibody of the invention, J695, was produced by site-directed mutagenesis of contact and hypermutation amino acids residues of antibody Y61 (see Example 2 and section III below). J695 differs from Y61 by a Gly to Tyr substitution in Y61 at position 50 of the light chain CDR2 and by a Gly to Tyr substitution at position 94 of the light chain CDR3. The heavy and light chain CDR3s of J695 are shown in SEQ ID NOs: 25 and 26, respectively, the heavy and light chain CDR2s of J695 are shown in SEQ ID NOs: 27 and 28, respectively, and the heavy and light chain CDR1s of J695 are shown in SEQ ID NOs: 29 and 30, respectively. The VH of J695 has the amino acid sequence of SEQ ID NO: 31 and the VL of J695 has the amino acid sequence of SEQ ID NO: 32 (these sequences are also shown in Figures 1A-1D, aligned with Joe9).

Accordingly, in another aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹ M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

In preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28.

In another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

5 In yet another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 32.

In certain embodiments, the full length antibody comprises a heavy chain
10 constant region, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions and any allotypic variant therein as described in Kabat (, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Preferably, the antibody heavy chain constant region is an IgG1 heavy chain constant region. Alternatively, the
15 antibody portion can be an Fab fragment, an F(ab')₂ fragment or a single chain Fv fragment. -

Additional mutations in the preferred consensus sequences for CDR3, CDR2, and CDR1 of antibodies on the lineage from Joe 9 to J695, or from the lineage Y61 to J695, can be made to provide additional anti-IL-12 antibodies of the invention. Such
20 methods of modification can be performed using standard molecular biology techniques, such as by PCR mutagenesis, targeting individual contact or hypermutation amino acid residues in the light chain and/or heavy chain CDRs-, followed by kinetic and functional analysis of the modified antibodies as described herein (e.g., neutralization assays described in Example 3, and by BIAcore analysis, as described in Example 5).

25 Accordingly, in another aspect the invention features an isolated human antibody, or an antigen-binding portion thereof, which

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁶ M or less;

b) comprises a heavy chain CDR3 comprising the amino acid sequence of
30 SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising
35 the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a mutant thereof having one or more amino acid substitutions at a preferred selective
 5 mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6.

10 In another aspect the invention features an isolated human antibody, or an antigen-binding portion thereof, which

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of 1×10^{-9} M or less;

b) comprises a heavy chain CDR3 comprising the amino acid sequence of
 15 SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a
 20 heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID
 25 NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain
 30 CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

An ordinarily skilled artisan will also appreciate that additional mutations to the CDR regions of an antibody of the invention, for example in Y61 or in J695, can be made to provide additional anti-IL-12 antibodies of the invention. Such methods of
 35 modification can be performed using standard molecular biology techniques, as described above. The functional and kinetic analysis of the modified antibodies can be performed as described in Example 3 and Example 5, respectively. Modifications of

individual residues of Y61 that led to the identification of J695 are shown in Figures 2A-2H and are described in Example 2.

Accordingly, in another aspect the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- 5 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1×10^{-9} M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 10 21, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19, and a heavy chain CDR1 15 comprising the amino acid sequence of SEQ ID NO: 21; and
- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22, or a mutant thereof having one or more amino acid substitutions at a preferred 20 selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.
- 25 In another aspect the invention features an isolated human antibody, or an antigen-binding portion thereof, which
- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1×10^{-9} M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of 30 SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 35 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

In yet another embodiment, the invention provides isolated human antibodies, or antigen-binding portions thereof, that neutralize the activity of human IL-12, and at least one additional primate IL-12 selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-12, cynomolgus IL-12 and rhesus IL-12, but which do not neutralize the activity of the mouse IL-12.

II Selection of Recombinant Human Antibodies

Recombinant human antibodies of the invention can be isolated by screening of a recombinant combinatorial antibody library, preferably a scFv phage display library, prepared using human VL and VH cDNAs prepared from mRNA derived from human lymphocytes. Methodologies for preparing and screening such libraries are known in the art. In addition to commercially available kits for generating phage display libraries (e.g., the Pharmacia *Recombinant Phage Antibody System*, catalog no. 27-9400-01; and the Stratagene *SurfZAP*TM phage display kit, catalog no. 240612), examples of methods and reagents particularly amenable for use in generating and screening antibody display libraries can be found in, for example, Kang *et al.* PCT Publication No. WO 92/18619; Winter *et al.* PCT Publication No. WO 92/20791; Breitling *et al.* PCT Publication No. WO 93/01288; McCafferty *et al.* PCT Publication No. WO 92/01047; Garrard *et al.* PCT Publication No. WO 92/09690; Fuchs *et al.* (1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3576-3580; Garrard *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982.

The antibody libraries used in this method are preferably scFv libraries prepared from human VL and VH cDNAs. The scFv antibody libraries are preferably screened using recombinant human IL-12 as the antigen to select human heavy and light chain sequences having a binding activity toward IL-12. To select for antibodies specific for

the p35 subunit of IL-12 or the p70 heterodimer, screening assays were performed in the presence of excess free p40 subunit. Subunit preferences can be determined, for example by, micro-Friguet titration, as described in Example 1.

Once initial human VL and VH segments are selected, "mix and match" experiments, in which different pairs of the selected VL and VH segments are screened for IL-12 binding, are performed to select preferred VL/VH pair combinations (see Example 1). Additionally, to further improve the affinity and/or lower the off rate constant for hIL-12 binding, the VL and VH segments of the preferred VL/VH pair(s) can be randomly mutated, preferably within the CDR3 region of VH and/or VL, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. This *in vitro* affinity maturation can be accomplished by amplifying VH and VL regions using PCR primers complimentary to the VH CDR3 or VL CDR3, respectively, which primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode VH and VL segments into which random mutations have been introduced into the VH and/or VL CDR3 regions. These randomly mutated VH and VL segments can be reselected and rescreened for binding to hIL-12 and sequences that exhibit high affinity and a low off rate for IL-12 binding can be selected. Table 2 (see Appendix A) shows antibodies that displayed altered binding specificity/affinity produced as a result of *in vitro* affinity maturation.

Following selection, isolation and screening of an anti-hIL-12 antibody of the invention from a recombinant immunoglobulin display library, nucleic acid encoding the selected antibody can be recovered from the phage particle(s) (*e.g.*, from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can be further manipulated to create other antibody forms of the invention (*e.g.*, linked to nucleic acid encoding additional immunoglobulin domains, such as additional constant regions). To express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into a mammalian host cells, as described in further detail in Section IV below.

Methods for selecting human IL-12 binding antibodies by phage display technology, and affinity maturation of selected antibodies by random or site-directed mutagenesis of CDR regions are described in further detail in Example 1.

As described in Example 1, screening of human VL and VH cDNA libraries identified a series of anti-IL-12 antibodies, of which the Joe 9 antibody was selected for further development. A comparison of the heavy chain variable region of Joe 9 with the heavy chain germline sequences selected from the VBASE database, revealed that Joe 9

was similar to the COS-3 germline sequence. COS-3 belongs to the V_H3 family of germline sequences.

The V_H3 family is part of the human VH germline repertoire which is grouped into seven families, V_H1-V_H7, based on nucleotide sequence homology (Tomlinson *et al.* (1992) *J. Mol. Biol.*, 227, 776-798 and Cook *et al.* (1995) *Immunology Today*, 16, 237-242). The V_H3 family contains the highest number of members and makes the largest contribution to the germline repertoire. For any given human V_H3- germline antibody sequence, the amino acid sequence identity within the entire V_H3 family is high (See *e.g.*, Tomlinson *et al.* (1992) *J. Mol. Biol.*, 227, 776-798 and Cook *et al.* (1995) *Immunology Today*, 16, 237-242). The range of amino acid sequence identity between any two germline VH sequences of the V_H3 family varies from 69-98 residues out of approximately 100 VH residues, (*i.e.*, 69-98% amino acid sequence homology between any two germline VH sequences). For most pairs of germline sequences there is at least 80 or more identical amino acid residues, (*i.e.*, at least 80% amino acid sequence homology). The high degree of amino acid sequence homology between the V_H3 family members results in certain amino acid residues being present at key sites in the CDR and framework regions of the VH chain. These amino acid residues confer structural features upon the CDRs.

Studies of antibody structures have shown that CDR conformations can be grouped into families of canonical CDR structures based on the key amino acid residues that occupy certain positions in the CDR and framework regions. Consequently, there are similar local CDR conformations in different antibodies that have canonical structures with identical key amino acid residues (Chothia *et al.* (1987) *J. Mol. Biol.*, 196, 901-917 and Chothia *et al.* (1989) *Nature*, 342, 877-883). Within the V_H3 family there is a conservation of amino acid residue identity at the key sites for the CDR1 and CDR2 canonical structures (Chothia *et al.* (1992) *J. Mol. Biol.*, 227, 799-817).

The COS-3 germline VH gene, is a member of the V_H3 family and is a variant of the 3-30 (DP-49) germline VH allele. COS-3, differs from Joe9 VH amino acid sequences at only 5 positions. The high degree of amino acid sequence homology between Joe9 VH and COS-3, and between Joe9 VH and the other V_H3 family members also confers a high degree of CDR structural homology (Chothia *et al.* (1992) *J. Mol. Biol.*, 227, 799-817; Chothia *et al.* (1987) *J. Mol. Biol.*, 196, 901-917 and Chothia *et al.* (1989) *Nature*, 342, 877-883).

The skilled artisan will appreciate that based on the high amino acid sequence and canonical structural similarity to Joe 9, other V_H3 family members could also be used to generate antibodies that bind to human IL-12. This can be performed, for example, by selecting an appropriate VL by chain-shuffling techniques (Winter *et al.* (1994) *Annual Rev. Immunol.*, 12, 433-55), or by the grafting of CDRs from a rodent or

other human antibody including CDRs from antibodies of this invention onto a V_H3 family framework.

The human V lambda germline repertoire is grouped into 10 families based on nucleotide sequence homology (Williams *et al.* (1996) *J. Mol. Biol.*, 264, 220-232). A comparison of the light chain variable region of Joe 9 with the light chain germline sequences selected from the VBASE database, revealed that Joe 9 was similar to the DPL8 lambda germline. The Joe9 VL differs from DPL8 sequence at only four framework positions, and is highly homologous to the framework sequences of the other $V_{\lambda}1$ family members. Based on the high amino acid sequence homology and canonical structural similarity to Joe 9, other $V_{\lambda}1$ family members may also be used to generate antibodies that bind to human IL-12. This can be performed, for example, by selecting an appropriate VH by chain-shuffling techniques (Winter *et al. Supra*, or by the grafting of CDRs from a rodent or other human antibody including CDRs from antibodies of this invention onto a $V_{\lambda}1$ family framework.

The methods of the invention are intended to include recombinant antibodies that bind to hIL-12, comprising a heavy chain variable region derived from a member of the V_H3 family of germline sequences, and a light chain variable region derived from a member of the $V_{\lambda}1$ family of germline sequences. Moreover, the skilled artisan will appreciate that any member of the V_H3 family heavy chain sequence can be combined with any member of the $V_{\lambda}1$ family light chain sequence.

Those skilled in the art will also appreciate that DNA sequence polymorphisms that lead to changes in the amino acid sequences of the germline may exist within a population (e.g., the human population). Such genetic polymorphism in the germline sequences may exist among individuals within a population due to natural allelic variation. Such natural allelic variations can typically result in 1-5 % variance in the nucleotide sequence of the a gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in germline sequences that are the result of natural allelic variation are intended to be within the scope of the invention.

Accordingly, in one aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.
- b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the V_H3 germline family, wherein the heavy chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

In another preferred embodiment, the isolated human antibody, or antigen binding has mutation in the light chain CDR3.

In another preferred embodiment, the isolated human antibody, or antigen binding has mutation in the light chain CDR2.

15 In another preferred embodiment, the isolated human antibody, or antigen binding has mutation in the light chain CDR1.

An ordinarily skilled artisan will appreciate that based on the high amino acid sequence similarity between members of the V_H3 germline family, or between members of the light chain V_λ1 germline family, that mutations to the germlines sequences can provide additional antibodies that bind to human IL-12. Table 1 (see Appendix A) shows the germline sequences of the V_H3 family members and demonstrates the significant sequence homology within the family members. Also shown in Table 1 are the germline sequences for V_λ1 family members. The heavy and light chain sequences of Joe 9 are provided as a comparison. Mutations to the germline sequences of V_H3 or V_λ1 family members may be made, for example, at the same amino acid positions as those made in the antibodies of the invention (e.g. mutations in Joe 9). The modifications can be performed using standard molecular biology techniques, such as by PCR mutagenesis, targeting individual amino acid residues in the germline sequences, followed by kinetic and functional analysis of the modified antibodies as described herein (e.g., neutralization assays described in Example 3, and by BIAcore analysis, as described in Example 5).

35 a) has a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 595-667, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

b) has a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 669-675, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

5 An ordinarily skilled artisan will appreciate that based on the high amino acid sequence similarity between Joe 9 and COS-3 heavy chain germline sequence, and between Joe 9 and DPL8 lambda germline sequence, that other mutations to the CDR regions of these germlines sequences can provide additional antibodies that bind to human IL-12. Such methods of modification can be performed using standard molecular
10 biology techniques as described above.

Accordingly, in one aspect, the invention features isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1s^{-1} or less, as determined by surface plasmon resonance, or which
15 inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6}\text{M}$ or less.

b) has a heavy chain variable region comprising the COS-3 germline amino acid sequence, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an
20 activity enhancing amino acid residue.

c) has a light chain variable region comprising the DPL8 germline amino acid sequence, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

25 Due to certain amino acid residues occupying key sites in the CDR and framework regions in the light and heavy chain variable region, structural features are conferred at these regions. In particular, the CDR2 and CDR1 regions are subject to canonical structural classifications. Since there is a high degree of amino acids sequence homology between family members, these canonical features are present between family
30 members. The skilled artisan will appreciate that modifications at the amino acid residues that confer these canonical structures would produce additional antibodies that bind to IL-12. The modifications can be performed using standard molecular biology techniques as described above.

Accordingly, in another aspect, the invention features an isolated human
35 antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1s^{-1} or less, as determined by surface plasmon resonance, or which

inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6}M$ or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the V_H3 germline family, wherein the heavy chain variable region comprises a CDR2 that is structurally similar to CDR2s from other V_H3 germline family members, and a CDR1 that is structurally similar to CDR1s from other V_H3 germline family members, and wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue;

c) has a light chain variable region comprising an amino acid sequence selected from a member of the V_L1 germline family, wherein the light chain variable region comprises a CDR2 that is structurally similar to CDR2s from other V_L1 germline family members, and a CDR1 that is structurally similar to CDR1s from other V_L1 germline family members, and wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

Recombinant human antibodies of the invention have variable and constant regions which are homologous to human germline immunoglobulin sequences selected from the VBASE database. Mutations to the recombinant human antibodies (*e.g.*, by random mutagenesis or PCR mutagenesis) result in amino acids that are not encoded by human germline immunoglobulin sequences. Also, libraries of recombinant antibodies which were derived from human donors will contain antibody sequences that differ from their corresponding germline sequences due to the normal process of somatic mutation that occurs during B-cell development. It should be noted that if the "germline" sequences obtained by PCR amplification encode amino acid differences in the framework regions from the true germline configuration (*i.e.*, differences in the amplified sequence as compared to the true germline sequence), it may be desirable to change these amino acid differences back to the true germline sequences (*i.e.*, "backmutation" of framework residues to the germline configuration). Thus, the present invention can optionally include a backmutation step. To do this, the amino acid sequences of heavy and light chain encoded by the germline (as found as example in VBASE database) are first compared to the mutated immunoglobulin heavy and light chain framework amino acid sequences to identify amino acid residues in the mutated immunoglobulin framework sequence that differ from the closest germline sequences. Then, the appropriate nucleotides of the mutated immunoglobulin sequence are mutated back to correspond to the germline sequence, using the genetic code to determine which nucleotide changes should be made. Mutagenesis of the mutated immunoglobulin framework sequence is carried out by standard methods, such as PCR-mediated

mutagenesis (in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product contains the mutations) or site-directed mutagenesis. The role of each amino acid identified as candidate for backmutation should be investigated for a direct or indirect role in antigen binding and any amino acid found after mutation to affect any desirable characteristic of the human antibody should not be included in the final human antibody; as an example, activity enhancing amino acids identified by the selective mutagenesis approach will not be subject to backmutation. Assays to determine the characteristics of the antibody resulting from mutagenesis can include ELISA, competitive ELISA, *in vitro* and *in vivo* neutralization assays and/or (see e.g. Example 3) immunohistochemistry with tissue sections from various sources (including human, primate and/or other species).

To minimize the number of amino acids subject to backmutation those amino acid positions found to be different from the closest germline sequence but identical to the corresponding amino acid in a second germline sequence can remain, provided that the second germline sequence is identical and colinear to the sequence of the human antibody of the invention for at least 10, preferably 12 amino acids, on both sides of the amino acid in question. This would assure that any peptide epitope presented to the immune system by professional antigen presenting cells in a subject treated with the human antibody of the invention would not be foreign but identical to a self-antigen, i.e. the immunoglobulin encoded by that second germline sequence. Backmutation may occur at any stage of antibody optimization; preferably, backmutation occurs directly before or after the selective mutagenesis approach. More preferably, backmutation occurs directly before the selective mutagenesis approach.

III. Modifications to Preferred Selective Mutagenesis Positions, Contact and/or Hypermutation Positions

Typically, selection of antibodies with improved affinities can be carried out using phage display methods, as described in section II above. This can be accomplished by randomly mutating combinations of CDR residues and generating large libraries containing antibodies of different sequences. However, for these selection methods to work, the antibody-antigen reaction must tend to equilibrium to allow, over time, preferential binding of higher affinity antibodies to the antigen. Selection conditions that would allow equilibrium to be established could not be determined (presumably due to additional non-specific interactions between the antigen and phage particle) when phage display methods were used to improve the affinity of selected anti-IL-12 antibodies, upon attaining a certain level of affinity achieved (*i.e.*, that of antibody Y61). Accordingly, antibodies with even higher affinities could not be selected by phage display methods. Thus, for at least certain antibodies or antigens, phage display

methods are limiting in their ability to select antibodies with a highly improved binding specificity/affinity. Accordingly, a method termed Selective Mutagenesis Approach which does not require phage display affinity maturation of antibodies, was established to overcome this limitation and is provided by the invention. Although this Selective

5 Mutagenesis Approach was developed to overcome limitations using the phage display system, it should be noted that this method can also be used with the phage display system. Moreover, the selective mutagenesis approach can be used to improve the activity of any antibody.

To improve the activity (e.g., affinity or neutralizing activity) of an antibody,

10 ideally one would like to mutate every CDR position in both the heavy and light chains to every other possible amino acid residue. However, since there are, on average, 70 CDR positions within an antibody, such an approach would be very time consuming and labor intensive. Accordingly, the method of the invention allows one to improve the activity of the antibody by mutating only certain selected residues within the heavy

15 and/or light chain CDRs. Furthermore, the method of the invention allows improvement in activity of the antibody without affecting other desirable properties of the antibody.

Determining which amino acid residues of an antibody variable region are in contact with an antigen cannot be accurately predicted based on primary sequence or their positions within the variable region. Nevertheless, alignments of sequences from

20 antibodies with different specificities conducted by Kabat *et al.* have identified the CDRs as local regions within the variable regions which differ significantly among antibodies (Kabat *et al.* (1971) *Ann. NY Acad. Sci.* 190:382-393, , Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Structural studies have

25 shown that the antigen binding surface is formed by amino acid residues present in the CDRs. Other amino acid residues outside the CDR are also known to play structural roles or be directly involved in antigen binding. Therefore, for each antigen-antibody pair, amino acid residues within and outside of the CDRs may be important.

The sequence alignment studies by Tomlison *et al* identified a number of

30 positions in the heavy and light chain CDR1 and CDR2, and in a portion of the kappa chain CDR3 which are frequent sites of somatic mutation. (Tomlison *et al* (1996) *J. Mol. Biol.* 256: 813-817). In particular, positions H31, H31B, H33, H33B, H52B, H56, H58, L30, L31, L31A, L50, L53, L91, L92, L93 and L94 were identified as frequent sites for somatic mutation. However, this analysis excludes the important heavy chain

35 CDR3 regions, and sections of the light chain CDR3 which are known to lie in the center of an antibody binding site, and potentially provide important interactions with an antigen. Furthermore, Tomlison *et al.* propose that somatic diversity alone does not necessarily predict a role of a specific amino acid in antigen binding, and suggest

conserved amino acid residues that contact the antigen, and diverse amino acid residues which do not contact the antigen. This conclusion is further supported by mutational studies on the role of somatic mutations to antibody affinity (*Sharon, (1990), PNAS, 87:4814-7*). Nineteen somatic mutations in a high-affinity anti-p-azophenylarsonate (Ars) antibody were simultaneously replaced with their corresponding germline residues, generating a germline version of the anti-Ars antibody which had a two-hundred fold loss in activity. The full affinity of the anti-Ars antibody could be recovered by restoring only three of the nineteen somatic mutations, demonstrating that many somatic mutations may be permitted that do not contribute to antigen binding activity.

The result can be explained in part by the nature of antibody diversity itself. Immature B-cells may produce initially low affinity antibodies that recognize a number of self or non-self antigens. Moreover, antibodies may undergo in the course of affinity maturation sequence variations that may cause self-reactivity. Hypermutation of such low affinity antibodies may serve to abolish self-reactivity ("negative selection") and increase affinity for the foreign antigen. Therefore, the analysis of primary and structural data of a large number of antibodies does not provide a method of predicting either (1) the role of somatic hyper-mutation sites in the affinity maturation process versus the process of decreasing affinity towards unwanted antigens, or (2) how a given amino acid contributes to the properties of a specific antigen-antibody pair.

Other attempts to address the role of specific amino acid residues in antigen recognition were made by analyzing a number of crystal structures of antigen-antibody complexes (*MacCallum et al. (1996) J. Mol. Biol. 262: 732-745*). The potential role of positions located within and outside the CDRs was indicated. Positions in CDRs involved in antigen binding in more than 10 of 26 analyzed structures included H31, H33, H50, H52, H53, H54, H56, H58, H95, H96, H97, H98 and H100 in the heavy chain and L30A, L32, L91, L92, L93, L94, L96 in the light chain. However, the authors noted that prediction of antigen contacts using these and other structural data may over and under predict contact positions, leading to the speculation that a different strategy may have to be applied to different antigens.

Pini *et al.* describe randomizing multiple residues in antibody CDR sequences in a large phage display library to rapidly increase antibody affinity (*Pini et al. (1998) J. Biol Chem. 273: 21769-21776*). However, the high affinity antibodies discussed by Pini *et al.* had mutations in a total of eight positions, and a reductionary analysis of which changes are absolutely required to improve affinity of the antibody becomes impractical because of the large number of possible combinations to be tested for the smallest number of amino acids required.

Furthermore, randomizing multiple residues may not necessarily preserve other desired properties of the antibody. Desirable properties or characteristics of an antibody are art-recognized and include for example, preservation of non-cross reactivity, e.g., with other proteins or human tissues and preservation of antibody sequences that are close to human germline immunoglobulin sequences improvement of neutralization potency. Other desirable properties or characteristics include ability to preserve species cross reactivity, ability to preserve epitope specificity and ability to preserve high expression levels of protein in mammalian cells. The desirable properties or characteristics can be observed or measured using art-recognized techniques including but not limited to ELISA, competitive ELISA, *in vitro* and *in vivo* neutralization assays (see e.g. Example 3), immunohistochemistry with tissue sections from different sources including human, primate or other sources as the need may be, and studies to expression in mammalian cells using transient expression or stable expression.

In addition, the method of Pini et al may introduce more changes than the minimal number actually required to improve affinity and may lead to the antibodies triggering anti-human-antibody (HAMA) formation in human subjects. Further, as discussed elsewhere, the phage display as demonstrated here, or other related method including ribosome display may not work appropriately upon reaching certain affinities between antibody and antigen and the conditions required to reach equilibrium may not be established in a reasonable time frame because of additional interactions including interactions with other phage or ribosome components and the antigen.

The ordinarily skilled artisan may glean interesting scientific information on the origin of antibody diversity from the teachings of the references discussed above. The present invention, however, provides a method for increasing antibody affinity of a specific antigen-antibody pair while preserving other relevant features or desirable characteristics of the antibody. This is especially important when considering the desirability of imparting a multitude of different characteristics on a specific antibody including antigen binding.

If the starting antibody has desirable properties or characteristics which need to be retained, a selective mutagenesis approach can be the best strategy for preserving these desirable properties while improving the activity of the antibody. For example, in the mutagenesis of Y61, the aim was to increase affinity for hIL-12, and to improve the neutralization potency of the antibody while preserving desired properties. Desired properties of Y61 included (1) preservation of non-cross reactivity with other proteins or human tissues, (2) preservation of fine epitope specificity, i.e. recognizing a p40 epitope preferably in the context of the p70 (p40/p35) heterodimer, thereby preventing binding interference from free soluble p40; and (3) generation of an antibody with heavy and

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light chain amino acid sequences that were as close as possible to their respective germline immunoglobulin sequences.

In one embodiment, the method of the invention provides a selective mutagenesis approach as a strategy for preserving the desirable properties or characteristics of the antibody while improving the affinity and/or neutralization potency. The term "selective mutagenesis approach" is as defined above and includes a method of individually mutating selected amino acid residues. The amino acid residues to be mutated may first be selected from preferred selective mutagenesis positions, then from contact positions, and then from hypermutation positions. The individual selected position can be mutated to at least two other amino acid residue and the effect of the mutation both on the desired properties of the antibody, and improvement in antibody activity is determined.

The Selective Mutagenesis approach comprises the steps of:

selecting candidate positions in the order 1) preferred selective mutagenesis positions; 2) contact positions; 3) hypermutation positions and ranking the positions based on the location of the position within the heavy and light chain variable regions of an antibody (CDR3 preferred over CDR2 preferred over CDR1);

individually mutating candidate preferred selective mutagenesis positions, hypermutation and/or contact positions in the order of ranking, to all possible other amino acid residues and analyzing the effect of the individual mutations on the activity of the antibody in order to determine activity enhancing amino acid residues;

if necessary, making stepwise combinations of the individual activity enhancing amino acid residues and analyzing the effect of the various combinations on the activity of the antibodies; selecting mutant antibodies with activity enhancing amino acid residues and ranking the mutant antibodies based on the location and identity of the amino acid substitutions with regard to their immunogenic potential. Highest ranking is given to mutant antibodies that comprise an amino acid sequence which nearly identical to a variable region sequence that is described in a germline database, or has an amino acid sequence that is comparable to other human antibodies. Lower ranking is given to mutant antibodies containing an amino acid substitution that is rarely encountered in either germline sequences or the sequences of other human antibodies. The lowest ranking is given to mutant antibodies with an amino acid substitution that has not been encountered in a germline sequence or the sequence of another human antibody. As set forth above, mutant antibodies comprising at least one activity enhancing amino acid residue located in CDR3 is preferred over CDR2 which is preferred over CDR1. The CDRs of the heavy chain variable regions are preferred over those of the light chain variable region.

The mutant antibodies can also be studied for improvement in activity, *e.g.* when compared to their corresponding parental antibody. The improvement in activity of the mutant antibody can be determined for example, by neutralization assays, or binding specificity/affinity by surface plasmon resonance analysis (see Example 3). Preferably, the improvement in activity can be at least 2-20 fold higher than the parental antibody. The improvement in activity can be at least " x_1 " to " x_2 " fold higher than the parental antibody wherein " x_1 " and " x_2 " are integers between and including 2 to 20, including ranges within the state range, *e.g.* 2-15, *e.g.* 5-10.

The mutant antibodies with the activity enhancing amino acid residue also can be studied to determine whether at least one other desirable property has been retained after mutation. For example, with anti-hIL-12 antibodies testing for, (1) preservation of non-cross reactivity with other proteins or human tissues, (2) preservation of epitope recognition, *i.e.* recognizing a p40 epitope preferably in the context of the p70 (p40/p35) heterodimer, thereby preventing binding interference from free soluble p40; and (3) generation of antibodies with heavy and light chain amino acid sequences that were as close as possible to their respective germline immunoglobulin sequences, and determining which would be least likely to elicit a human immune response based on the number of differences from the germline sequence. The same observations can be made on an antibody having more than one activity enhancing amino acid residues, *e.g.* at least two or at least three activity enhancing amino acid residues, to determine whether retention of the desirable property or characteristic has occurred.

An example of the use of a "selective mutagenesis approach", in the mutagenesis of Y61 is described below. The individual mutations H31S→E, L50→Y, or L94G→Y each improved neutralization activity of the antibody. However, when combination clones were tested, the activity of the combined clone H31S→E + L50→Y + L94G→Y was no better than L50→Y + L94G→Y (J695). Therefore, changing the germline amino acid residue Ser to Glu at position 31 of CDR1 was unnecessary for the improved activity of J695 over Y61. The selective mutagenesis approach therefore, identified the minimal number of changes that contributed to the final activity, thereby reducing the immunogenic potential of the final antibody and preserving other desired properties of the antibody.

Isolated DNA encoding the VH and VL produced by the selected mutagenesis approach can be converted into full length antibody chain genes, to Fab fragment genes as to a scFV gene, as described in section IV. For expression of VH and VL regions produced by the selected mutagenesis approach, expression vectors encoding the heavy and light chain can be transfected into variety host cells as described in detail in section IV. Preferred host cells include either prokaryotic host cells, for example, *E. coli*, or

eukaryotic host cells, for example, yeast cells, e.g., *S. cerevisiae*. Most preferred eukaryotic host cells are mammalian host cells, described in detail in section IV.

The selective mutagenesis approach provides a method of producing antibodies with improved activities without prior affinity maturation of the antibody by other means. The selective mutagenesis approach provides a method of producing antibodies with improved affinities which have been subject to back mutations. The selective mutagenesis approach also provides a method of improving the activity of affinity matured antibodies.

The skilled artisan will recognize that the selective mutagenesis approach can be used in standard antibody manipulation techniques known in the art. Examples include, but are not limited to, CDR grafted antibodies, chimeric antibodies, scFV fragments, Fab fragments of a full length antibodies and human antibodies from other sources, e.g., transgenic mice.

Rapid large scale mutational analysis of antibodies include *in vitro* transcription and translation using ribosome display technology (see e.g., Hanes *et al.*, (1997) *Proc. Natl. Acad. Sci.* 94: 4937-4942; Dall Acqua *et al.*, (1998) *Curr. Opin. Struc. Biol.* 8: 443-450; He *et al.*, (1997) *Nucleic Acid Res.* 25: 5132-5134), and U.S. Patent Nos. 5,643,768 and 5,658,754 issued to Kawasaki. The selective mutagenesis approach also provides a method of producing antibodies with improved activities that can be selected using ribosomal display techniques.

In the methods of the invention, antibodies or antigen binding portions thereof are further modified by altering individual positions in the CDRs of the HCVR and/or LCVR. Although these modifications can be made in phage-displayed antibodies, the method is advantageous in that it can be performed with antibodies that are expressed in other types of host systems, such as bacterial, yeast or mammalian cell expression systems. The individual positions within the CDRs selected for modification are based on the positions being a contact and/or hypermutation position.

Preferred contact positions and hypermutation positions as defined herein are shown in Table 3 (see Appendix A) and their modification in accordance with the method of the invention is described in detail in Example 2. Preferred contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. Preferred hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93. More preferred amino acid residues (referred to as "preferred selective mutagenesis positions") are both contact and hypermutation positions and are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30,

L31, L32, L50, L91, L92, L93, L94. Particularly preferred contact positions are selected from the group consisting of L50 and L94.

Preferred activity enhancing amino acid residues replace amino acid residues located at positions selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94, and L96. More preferred activity enhancing amino acid residues replace amino acid residues located at positions H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94. Particularly, preferred activity enhancing amino acid residues replace amino acid residues located at positions selected from the group consisting of L50 and L94.

In general, the method of the invention involves selecting a particular preferred selective mutagenesis position, contact and/or hypermutation position within a CDR of the heavy or light chain of a parent antibody of interest, or antigen binding portion thereof, randomly mutagenizing that individual position (e.g., by genetic means using a mutagenic oligonucleotide to generate a "mini-library" of modified antibodies), or mutating a position to specific desired amino acids, to identify activity enhancing amino acid residues expressing, and purifying the modified antibodies (e.g., in a non-phage display host system), measuring the activity of the modified antibodies for antigen (e.g., by measuring k_{off} rates by BIAcore analysis), repeating these steps for other CDR positions, as necessary, and combining individual mutations shown to have improved activity and testing whether the combination(s) generate an antibody with even greater activity (e.g., affinity or neutralizing potency) than the parent antibody, or antigen-binding portion thereof.

Accordingly, in one embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting in order a 1) preferred selective mutagenesis position, 2) contact position, or 3) hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;
- e) optionally, repeating steps a) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position;

f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained. Preferably, the selected antibody or antibodies have an improved activity without loss or with retention of at least one desirable characteristic or property of the parental antibody as described above. The desirable characteristic or property can be measured or observed by the ordinarily skilled artisan using art-recognized techniques.

Preferred contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. Preferred hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93. More preferred preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94. Particularly preferred contact positions are selected from the group consisting of L50 and L94.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;
- e) optionally, repeating steps a) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position;
- f) combining, in the parent antibody, or antigen-binding portion thereof, two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and
- g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof;

until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferred contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. Preferred hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93. More preferred preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94. Particularly preferred contact positions are selected from the group consisting of L50 and L94.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
 - b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation;
 - c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
 - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;
 - e) optionally, repeating steps a) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position;
 - f) combining, in the parent antibody, or antigen-binding portion thereof, three individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and
 - g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof;
- until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the activity enhancing amino acid residue replaces amino acid residues located at positions selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, 35 L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96.

Following mutagenesis of individual selected positions, mutated clones can be sequenced to identify which amino acid residues have been introduced into the selected position in each clone. A small number of clones (e.g., about 24) can be selected for

sequencing, which statistically should yield 10-15 unique antibodies, whereas larger numbers of clones (e.g., greater than 60) can be sequenced to ensure that antibodies with every possible substitution at the selected position are identified.

In one embodiment, contact and/or hypermutation positions within the CDR3 regions of the heavy and/or light chains are first selected for mutagenesis. However, for antibodies that have already been affinity matured *in vitro* by random mutagenesis of the CDR3 regions via phage display selection, it may be preferably to first select contact and/or hypermutation positions within CDR1 or CDR2 of the heavy and/or light chain.

In a more preferred embodiment, preferred selective mutagenesis positions within the CDR3 regions of the heavy and/or light chains are first selected for mutagenesis. However, for antibodies that have already been affinity matured *in vitro* by random mutagenesis of the CDR3 regions via phage display selection, it may be preferably to first select preferred selective mutagenesis positions within CDR1 or CDR2 of the heavy and/or light chain.

In another preferred embodiment, the optimization of a selected antibody by the selective mutagenesis approach is done sequentially as follows: preferred selective mutagenesis positions selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 are mutated first to at least 2 other amino acids each (preferably 5-14 other amino acids) and the resulting antibodies are characterized for increased affinity, neutralization potency (and possibly also for at least one other retained characteristic or property discussed elsewhere). If a mutation of a single preferred selective mutagenesis position does not increase the affinity or neutralization potency at all or sufficiently and if even the combination of multiple activity enhancing amino acids replacing amino acids in preferred selective mutagenesis positions does not result in an combination antibody which meets the target activity (including affinity and/or neutralization potency), additional amino acid residues will be selected for selective mutagenesis from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 are mutated to at least 2 other amino acids each (preferably 5-14 other amino acids) and the resulting antibodies are characterized for increased affinity, neutralization potency (and possibly also for at least one other retained characteristic or property discussed elsewhere).

If a mutation of a single amino acid residue selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 does not increase the activity (including affinity and/or neutralization potency) at all or not sufficiently and if even the combination of multiple activity enhancing amino acids replacing amino acids in those positions does not result in an combination antibody which meets the targeted activity (including affinity and/or target neutralization potency), additional amino acid residues will be selected for selective mutagenesis from the group consisting of H33B, H52B,

L31A and are mutated to at least 2 other amino acids each (preferably 5-14 other amino acids) and the resulting antibodies are characterized for increased affinity, neutralization potency (and possibly also for at least one other retained characteristic or property discussed elsewhere).

- 5 It should be understood that the sequential selective mutagenesis approach may end at any of the steps outline above as soon as an antibody with the desired activity (including affinity and neutralization potency) has been identified. If mutagenesis of the preselected positions has identified activity enhancing amino acids residues but the combination antibody still do not meet the targets set for activity (including affinity and
10 neutralization potency) and/or if the identified activity enhancing amino acids also affect other desired characteristics and are therefore not acceptable, the remaining CDR residues may be subjected to mutagenesis (see section IV).

- The method of the invention can be used to improve activity of an antibody, or antigen binding portion thereof, to reach a predetermined target activity (e.g. a
15 predetermined affinity and/or neutralization potency, and/or a desired property or characteristic).

Accordingly, the invention provides a method of improving the activity of an antibody, or antigen-binding portion thereof, to attain a predetermined target activity, comprising:

- 20 a) providing a parent antibody a antigen-binding portion thereof;
b) selecting a preferred selective mutagenesis position selected from group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.
c) individually mutating the selected preferred selective mutagenesis position to
25 at least two other amino acid residues to hereby create a first panel of mutated antibodies, or antigen binding portions thereof;
d) evaluating the activity of the first panel of mutated antibodies, or antigen binding portions thereof to determined if mutation of a single selective mutagenesis position produces an antibody or antigen binding portion thereof with the
30 predetermined target activity or a partial target activity;
e) combining in a stepwise fashion, in the parent antibody, or antigen binding portion thereof, individual mutations shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof.
f) evaluating the activity of the combination antibodies, or antigen binding
35 portions thereof to determined if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity.
g) if steps d) or f) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result an antibody with only a partial

activity, additional amino acid residues selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 are mutated to at least two other amino acid residues to thereby create a second panel of mutated antibodies or antigen-binding portions thereof;

5 h) evaluating the activity of the second panel of mutated antibodies or antigen binding portions thereof, to determine if mutation of a single amino acid residue selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 results in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

10 i) combining in stepwise fashion in the parent antibody, or antigen-binding portion thereof, individual mutations of step g) shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof;

j) evaluating the activity of the combination antibodies or antigen binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity;

15 k) if steps h) or j) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result in an antibody with only a partial activity, additional amino acid residues selected from the group consisting of H33B, H52B and L31A are mutated to at least two other amino acid residues to thereby create a third panel of mutated antibodies or antigen binding portions thereof;

20 l) evaluating the activity of the third panel of mutated antibodies or antigen binding portions thereof, to determine if a mutation of a single amino acid residue selected from the group consisting of H33B, H52B and L31A resulted in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

m) combining in a stepwise fashion in the parent antibody, or antigen binding portion thereof, individual mutation of step k) shown to have an improved activity, to form combination antibodies, or antigen binding portions, thereof;

30 n) evaluating the activity of the combination antibodies or antigen-binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity to thereby produce an antibody or antigen binding portion thereof with a predetermined target activity.

A number of mutagenesis methods can be used, including PCR assembly, Kunkel (dut-ung-) and thiophosphate (Amersham Sculptor kit) oligonucleotide-directed mutagenesis.

A wide variety of host expression systems can be used to express the mutated antibodies, including bacterial, yeast, baculoviral and mammalian expression systems (as well as phage display expression systems). An example of a suitable bacterial

expression vector is pUC119(Sfi). Other antibody expression systems are known in the art and/or are described below in section IV.

The modified antibodies, or antigen binding portions thereof, produced by the method of the invention can be identified without the reliance on phage display methods for selection. Accordingly, the method of the invention is particularly advantageous for improving the activity of a recombinant parent antibody or antigen-binding portion thereof, that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in the phage-display system.

Accordingly, in another embodiment, the invention provides a method for improving the affinity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;
- e) optionally repeating steps b) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position;
- f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and
- g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferred contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. Preferred hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93. More preferred preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32,

H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94. Particularly preferred contact positions are selected from the group consisting of L50 and L94.

With available methods it is not possible or it is extremely laborious to derive an antibody with increased binding affinity and neutralization potency while retaining other properties or characteristics of the antibodies as discussed above. The method of this invention, however, can readily identify such antibodies. The antibodies subjected to the method of this invention can come from any source.

Therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof ;
 - b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;
 - c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expressing said panel in an appropriate expression system;
 - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;
 - e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristics, wherein the property or characteristic is one that needs to be retained in the antibody;
- until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and

L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

If therefore, the affinity of an antibody for a specific antigen should be improved, but where the phage display (or related system including ribosome display) method is no longer applicable, and other desirable properties or characteristics should be retained, the method of the invention can be used. Accordingly, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) optionally, repeating steps a) through e) for at least one other preferred selective mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained other property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94

and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35

heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;
- c) individually mutating said selected preferred selective mutagenesis positions, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) optionally, repeating steps a) through e) for at least one other preferred selective mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained other characteristic, to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding

interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1)

- 5 preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

10 IV. Modifications of other CDR residues

Ultimately, all CDR residues in a given antibody-antigen pair identified by any means to be required as activity enhancing amino acid residues and/or required directly or indirectly for binding to the antigen and/or for retaining other desirable properties or characteristics of the antibody. Such CDR residues are referred to as “preferred
15 selective mutagenesis positions”. It should be noted that in specific circumstances that preferred selective mutagenesis residues can be identified also by other means including co-crystallization of antibody and antigen and molecular modeling.

- If the preferred attempts to identify activity enhancing amino acids focussing on the preferred selective mutagenesis positions, contact or hypermutation positions
20 described above are exhausted, or if additional improvements are required, the remaining CDR residues may be modified as described below. It should be understood that the antibody could already be modified in any one or more contact or hypermutation positions according to the embodiments discussed above but may require further improvements. Therefore, in another embodiment, the invention provides a method for
25 improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53,
30 L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position e.g., to at least two other amino acid residues to thereby create a mutated antibody or a panel of mutated antibodies, or antigen-binding portions thereof;

- d) evaluating the activity of the mutated antibody or the panel of mutated
35 antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the mutated antibody or the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

If mutagenesis of a single residue is not sufficient other residues can be included; therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

If the preferred attempts to identify activity enhancing amino acids focussing on the contact or hypermutation positions described above are exhausted, or if additional

improvements are required, and the antibody in question can not further be optimized by mutagenesis and phage display (or related ribosome display) methods the remaining CDR residues may be modified as described below. It should be understood that the antibody could already be modified in any one or more preferred selective mutagenesis position, contact or hypermutation positions according to the embodiments discussed above but may require further improvements.

Therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and;
- c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic, until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

If a single mutagenesis is not sufficient to increase the affinity of the antibody other residues may be included in the mutagenesis. Therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 ;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity and other property or characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

The preferred attempts to identify activity enhancing amino acids focussing on the preferred selective mutagenesis positions, contact or hypermutation positions described may be exhausted, or additional improvements may be required, and it is important to retain other properties or characteristics of the antibody.

Therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, without affecting other characteristics, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

5 c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof
10 thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity and retained other property or characteristic, relative
15 to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce
20 an antibody with a close to germline immunoglobulin sequence

If mutagenesis of a single residue is not sufficient other residues can be included; therefore, in another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;
25 b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid
30 residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

35 e.) evaluating the panel of mutated antibodies or antigen-binding portions thereof, relative to the parent antibody or antigen-portion thereof, for changes in at least one other characteristic or property;

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e) repeating steps b) through e) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

5 f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and the retention of at least one other property or
10 characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained other property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

15 Mutagenesis of the preferred selective mutagenesis position, contact and hypermutation residues may not have increased the affinity of the antibody sufficiently, and mutagenesis and the phage display method (or related ribosome display method) may no longer be useful and at least one other characteristic or property of the antibody should be retained.

20 Therefore, in another embodiment the invention provides a method to improve the affinity of an antibody or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

25 b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

30 c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

35 e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion

thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope
 5 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

If mutagenesis of a single residue is not sufficient other residues can be included; therefore, in another embodiment, the invention provides a method for improving the
 10 activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region
 15 (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions
 20 thereof and expression in a non-phage display system;

d) evaluating the activity and retention of at least one other property or characteristic of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least
 30 two individual activity enhancing amino acid residues shown to have improved activity and not to affect at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and retention of at least one property or characteristic of the combination antibodies, or antigen-binding portions thereof with two activity
 35 enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one other retained characteristic or property, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

V. Expression of Antibodies

An antibody, or antibody portion, of the invention can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell.

- 5 To express an antibody recombinantly, a host cell is transfected with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such that the light and heavy chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant
- 10 DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Sambrook, Fritsch and Maniatis (eds), *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, N.Y., (1989), Ausubel, F.M. *et al.* (eds.) *Current Protocols in Molecular Biology*, Greene
- 15 Publishing Associates, (1989) and in U.S. Patent No. 4,816,397 by Boss *et al.*

- To obtain a DNA fragment encoding the heavy chain variable region of Joe 9 wt or a Joe 9 wt-related antibody, antibodies specific for human IL-12 were screened from human libraries and mutated, as described in section II. Once DNA fragments encoding Joe 9 wt or Joe 9 wt-related VH and VL segments are obtained, mutagenesis of these
- 20 sequences is carried out by standard methods, such as PCR site directed mutagenesis (PCR-mediated mutagenesis in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product contains the mutations) or other site-directed mutagenesis methods. Human IL-12 antibodies that displayed a level of activity and binding specificity/affinity that was desirable, for example J695, were further
 - 25 manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is
 - 30 intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

- The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences
- 35 of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification.

The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a lambda constant region.

To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence (Gly₄-Ser)₃, such that the VH and VL sequences can be expressed as a
20 contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, *Nature* (1990) 348:552-554).

To express the antibodies, or antibody portions of the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the J695 or J695-related light or heavy chain sequences, the expression vector may already carry antibody constant region sequences. For example, one approach to converting the J695 or J695-related VH and VL sequences to full-length

antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.*

Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see *e.g.*, U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell *et al.* and U.S. Patent No. 4,968,615 by Schaffner *et al.*, U.S. Patent No. 5,464,758 by Bujard *et al.* and U.S. Patent No. 5,654,168 by Bujard *et al.*

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see *e.g.*, U.S. Patents Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr⁻ host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody of this invention. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to hIL-12. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than hIL-12 by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to

enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows
5 for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are culture to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture
10 the host cells and recover the antibody from the culture medium. Antibodies or antigen-binding portions thereof of the invention can be expressed in an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L.D. *et al.* (1992) Nucl. Acids Res. 20: 6287-6295). Plant cells can also be modified to create transgenic plants that express the antibody or antigen binding portion thereof, of the invention.

15 In view of the foregoing, another aspect of the invention pertains to nucleic acid, vector and host cell compositions that can be used for recombinant expression of the antibodies and antibody portions of the invention. Preferably, the invention features isolated nucleic acids that encode CDRs of J695, or the full heavy and/or light chain variable region of J695. Accordingly, in one embodiment, the invention features an
20 isolated nucleic acid encoding an antibody heavy chain variable region that encodes the J695 heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25. Preferably, the nucleic acid encoding the antibody heavy chain variable region further encodes a J695 heavy chain CDR2 which comprises the amino acid sequence of SEQ ID NO: 27. More preferably, the nucleic acid encoding the antibody heavy chain
25 variable region further encodes a J695 heavy chain CDR1 which comprises the amino acid sequence of SEQ ID NO: 29. Even more preferably, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31 (the full VH region of J695).

In other embodiments, the invention features an isolated nucleic acid encoding
30 an antibody light chain variable region that encodes the J695 light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26. Preferably, the nucleic acid encoding the antibody light chain variable region further encodes a J695 light chain CDR2 which comprises the amino acid sequence of SEQ ID NO: 28. More preferably, the nucleic acid encoding the antibody light chain variable region further encodes a J695
35 light chain CDR1 which comprises the amino acid sequence of SEQ ID NO: 30. Even more preferably, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 32 (the full VL region of J695).

The invention also provides recombinant expression vectors encoding both an antibody heavy chain and an antibody light chain. For example, in one embodiment, the invention provides a recombinant expression vector encoding:

- a) an antibody heavy chain having a variable region comprising the amino acid sequence of SEQ ID NO: 31; and
- b) an antibody light chain having a variable region comprising the amino acid sequence of SEQ ID NO: 32.

The invention also provides host cells into which one or more of the recombinant expression vectors of the invention have been introduced. Preferably, the host cell is a mammalian host cell, more preferably the host cell is a CHO cell, an NS0 cell or a COS cell. Still further the invention provides a method of synthesizing a recombinant human antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant human antibody of the invention is synthesized. The method can further comprise isolating the recombinant human antibody from the culture medium.

VI. Pharmaceutical Compositions and Pharmaceutical Administration

The antibodies and antibody-portions of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody or antibody portion of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

The antibodies and antibody-portions of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate,

sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include 5 trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not 10 limited to polysorbate 20 and BRIJ surfactants.

In a preferred embodiment, the pharmaceutical composition includes the antibody at a dosage of about 0.01mg/kg - 10 mg/kg. More preferred dosages of the antibody include 1mg/kg administered every other week, or 0.3 mg/kg administered weekly.

15 The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the 20 form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by 25 intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active 30 compound (*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders 35 for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such

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as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

5 The antibodies and antibody-portions of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the
10 active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such
15 formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

 In certain embodiments, an antibody or antibody portion of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier.
20 The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the
25 invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

 Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody or antibody portion of the invention
30 is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders in which IL-12 activity is detrimental. For example, an anti-hIL-12 antibody or antibody portion of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind other cytokines or that bind cell surface
35 molecules). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various

monotherapies. It will be appreciated by the skilled practitioner that when the antibodies of the invention are used as part of a combination therapy, a lower dosage of antibody may be desirable than when the antibody alone is administered to a subject (e.g., a synergistic therapeutic effect may be achieved through the use of combination therapy which, in turn, permits use of a lower dose of the antibody to achieve the desired therapeutic effect).

Interleukin 12 plays a critical role in the pathology associated with a variety of diseases involving immune and inflammatory elements. These diseases include, but are not limited to, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, atopic dermatitis, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthropathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Acquired Immunodeficiency Related Diseases, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease,

rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus
 associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's
 disease associated lung disease, ankylosing spondylitis associated lung disease,
 vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced
 5 interstitial lung disease, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic
 pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease,
 gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical
 autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody
 hepatitis), autoimmune mediated hypoglycemia, type B insulin resistance with
 10 acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ
 transplantation, chronic immune disease associated with organ transplantation,
 osteoarthritis, primary sclerosing cholangitis, idiopathic leucopenia, autoimmune
 neutropenia, renal disease NOS, glomerulonephritides, microscopic vasculitis of the
 kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS,
 15 sperm autoimmunity, multiple sclerosis (all subtypes), insulin-dependent diabetes
 mellitus, sympathetic ophthalmia, pulmonary hypertension secondary to connective
 tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis
 nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis,
 Takayasu's disease/arteritis, autoimmune thrombocytopenia, idiopathic
 20 thrombocytopenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune
 hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary
 myxoedema, phacogenic uveitis, primary vasculitis and vitiligo. The human antibodies,
 and antibody portions of the invention can be used to treat autoimmune diseases, in
 particular those associated with inflammation, including, rheumatoid spondylitis,
 25 allergy, autoimmune diabetes, autoimmune uveitis.

Preferably, the antibodies of the invention or antigen-binding portions thereof,
 are used to treat rheumatoid arthritis, Crohn's disease, multiple sclerosis, insulin
 dependent diabetes mellitus and psoriasis, as described in more detail in section VII.

A human antibody, or antibody portion, of the invention also can be administered
 30 with one or more additional therapeutic agents useful in the treatment of autoimmune
 and inflammatory diseases.

Antibodies of the invention, or antigen binding portions thereof can be used
 alone or in combination to treat such diseases. It should be understood that the
 antibodies of the invention or antigen binding portion thereof can be used alone or in
 35 combination with an additional agent, e.g., a therapeutic agent, said additional agent
 being selected by the skilled artisan for its intended purpose. For example, the
 additional agent can be a therapeutic agent art-recognized as being useful to treat the
 disease or condition being treated by the antibody of the present invention. The

additional agent also can be an agent which imparts a beneficial attribute to the therapeutic composition e.g., an agent which effects the viscosity of the composition.

It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations which are part of this invention can be the antibodies of the present invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

Preferred combinations are non-steroidal anti-inflammatory drug(s) also referred to as NSAIDS which include drugs like ibuprofen. Other preferred combinations are corticosteroids including prednisolone; the well known side-effects of steroid use can be reduced or even eliminated by tapering the steroid dose required when treating patients in combination with the anti-IL-12 antibodies of this invention. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which an antibody, or antibody portion, of the invention can be combined include the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, or their ligands including CD154 (gp39 or CD40L).

Preferred combinations of therapeutic agents may interfere at different points in the autoimmune and subsequent inflammatory cascade; preferred examples include TNF antagonists like chimeric, humanized or human TNF antibodies, D2E7, (U.S. application serial number 08/599,226 filed February 9, 1996), cA2 (Remicade™), CDP 571, anti-TNF antibody fragments (e.g., CDP870), and soluble p55 or p75 TNF receptors, derivatives thereof, (p75TNFR1gG (Enbrel™) or p55TNFR1gG (Lenercept), soluble IL-13 receptor (sIL-13), and also TNF α converting enzyme (TACE) inhibitors; similarly IL-1 inhibitors (e.g., Interleukin-1-converting enzyme inhibitors, such as Vx740, or IL-1RA etc.) may be effective for the same reason. Other preferred combinations include Interleukin 11, anti-P7s and p-selectin glycoprotein ligand (PSGL). Yet another preferred combination are other key players of the autoimmune response which may act parallel to, dependent on or in concert with IL-12 function; especially preferred are IL-18 antagonists including IL-18 antibodies or soluble IL-18 receptors, or IL-18 binding proteins. It has been shown that IL-12 and IL-18 have overlapping but distinct functions and a combination of antagonists to both may be most effective. Yet another preferred

combination are non-depleting anti-CD4 inhibitors. Yet other preferred combinations include antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2) including antibodies, soluble receptors or antagonistic ligands.

The antibodies of the invention, or antigen binding portions thereof, may also be
 5 combined with agents, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochlincine, corticosteroids (oral, inhaled and local injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and
 10 oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNF α or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors),
 15 IL-1 β converting enzyme inhibitors (e.g., Vx740), anti-P7s, p-selectin glycoprotein ligand (PSGL), TNF α converting enzyme (TACE) inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors and the derivatives
 20 p75TNFRIgG (EnbrelTM) and p55TNFRIgG (Lenercept), sIL-1RI, sIL-1RII, sIL-6R, soluble IL-13 receptor (sIL-13)) and antiinflammatory cytokines (e.g. IL-4, IL-10, IL-11, IL-13 and TGF β). Preferred combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

Non-limiting examples of therapeutic agents for inflammatory bowel disease
 25 with which an antibody, or antibody portion, of the invention can be combined include the following: budesonide; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxigenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 β monoclonal antibodies; anti-IL-6
 30 monoclonal antibodies; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4,
 35 CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands. The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone,

phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNF α or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1 β converting enzyme inhibitors (e.g., Vx740), anti-P7s, p-selectin glycoprotein

5 ligand (PSGL), TNF α converting enzyme inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptapurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R, soluble IL-13 receptor (sIL-13)) and antiinflammatory cytokines (e.g. IL-4, IL-10,

10 IL-11, IL-13 and TGF β).

Preferred examples of therapeutic agents for Crohn's disease in which an antibody or an antigen binding portion can be combined include the following: TNF antagonists, for example, anti-TNF antibodies, D2E7 (U.S. application serial number 08/599,226, filed February 9, 1996), cA2 (RemicadeTM), CDP 571, anti-TNF antibody

15 fragments (e.g., CDP870), TNFR-Ig constructs (p75TNFR IgG (EnbrelTM) and p55TNFR IgG (Lenercept)), anti-P7s, p-selectin glycoprotein ligand (PSGL), soluble IL-13 receptor (sIL-13), and PDE4 inhibitors. Antibodies of the invention or antigen binding portions thereof, can be combined with corticosteroids, for example, budenoside and dexamethasone. Antibodies of the invention or antigen binding portions thereof,

20 may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid and olsalazine, and agents which interfere with synthesis or action of proinflammatory cytokines such as IL-1, for example, IL-1 β converting enzyme inhibitors (e.g., Vx740) and IL-1ra. Antibodies of the invention or antigen binding portion thereof may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors 6-

25 mercaptopurines. Antibodies of the invention or antigen binding portions thereof, can be combined with IL-11.

Non-limiting examples of therapeutic agents for multiple sclerosis with which an antibody, or antibody portion, of the invention can be combined include the following: corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide;

30 cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon- β 1a (Avonex; Biogen); interferon- β 1b (Betaseron; Chiron/Berlex); Copolymer 1 (Cop-1; Copaxone; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabribine; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-

35 CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. The antibodies of the invention, or antigen binding portions thereof, may also be

combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNF α or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1 β converting enzyme inhibitors (e.g., Vx740), anti-P7s, p-selectin glycoprotein ligand (PSGL), TACE inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R, soluble IL-13 receptor (sIL-13)) and antiinflammatory cytokines (e.g. IL-4, IL-10, IL-13 and TGF β).

Preferred examples of therapeutic agents for multiple sclerosis in which the antibody or antigen binding portion thereof can be combined to include interferon- β , for example, IFN β 1a and IFN β 1b; copaxone, corticosteroids, IL-1 inhibitors, TNF inhibitors, and antibodies to CD40 ligand and CD80.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound

calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.01-20 mg/kg, more preferably 1-10 mg/kg, even more preferably 0.3-1 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

VII. Uses of the Antibodies of the Invention

Given their ability to bind to hIL-12, the anti-hIL-12 antibodies, or portions thereof, of the invention can be used to detect hIL-12 (*e.g.*, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The invention provides a method for detecting hIL-12 in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of the invention and detecting either the antibody (or antibody portion) bound to hIL-12 or unbound antibody (or antibody portion), to thereby detect hIL-12 in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

Alternative to labeling the antibody, hIL-12 can be assayed in biological fluids by a competition immunoassay utilizing rhIL-12 standards labeled with a detectable substance and an unlabeled anti-hIL-12 antibody. In this assay, the biological sample,

the labeled rhIL-12 standards and the anti-hIL-12 antibody are combined and the amount of labeled rhIL-12 standard bound to the unlabeled antibody is determined. The amount of hIL-12 in the biological sample is inversely proportional to the amount of labeled rhIL-12 standard bound to the anti-hIL-12 antibody.

5 The Y61 and J695 antibodies of the invention can also be used to detect IL-12 from species other than humans, in particular IL-12 from primates. For example, Y61 can be used to detect IL-12 in the cynomolgus monkey and the rhesus monkey. J695 can be used to detect IL-12 in the cynomolgus monkey, rhesus monkey, and baboon. However, neither antibody cross reacts with mouse or rat IL-12 (see Example 3,
10 subsection F).

 The antibodies and antibody portions of the invention are capable of neutralizing hIL-12 activity *in vitro* (see Example 3) and *in vivo* (see Example 4). Accordingly, the antibodies and antibody portions of the invention can be used to inhibit IL-12 activity, *e.g.*, in a cell culture containing hIL-12, in human subjects or in other mammalian
15 subjects having IL-12 with which an antibody of the invention cross-reacts (*e.g.* primates such as baboon, cynomolgus and rhesus). In a preferred embodiment, the invention provides an isolated human antibody, or antigen-binding portion thereof, that neutralizes the activity of human IL-12, and at least one additional primate IL-12 selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-12,
20 cynomolgus IL-12 and rhesus IL-12, but which does not neutralize the activity of the mouse IL-12. Preferably, the IL-12 is human IL-12. For example, in a cell culture containing, or suspected of containing hIL-12, an antibody or antibody portion of the invention can be added to the culture medium to inhibit hIL-12 activity in the culture.

 In another embodiment, the invention provides a method for inhibiting IL-12
25 activity in a subject suffering from a disorder in which IL-12 activity is detrimental. IL-12 has been implicated in the pathophysiology of a wide variety of disorders (Windhagen *et al.*, (1995) *J. Exp. Med.* 182: 1985-1996; Morita *et al.* (1998) *Arthritis and Rheumatism.* 41: 306-314; Bucht *et al.*, (1996) *Clin. Exp. Immunol.* 103: 347-367; Fais *et al.* (1994) *J. Interferon Res.* 14:235-238; Parronchi *et al.*, (1997) *Am. J. Path.*
30 150:823-832; Monteleone *et al.*, (1997) *Gastroenterology.* 112:1169-1178, and Berrebi *et al.*, (1998) *Am. J. Path.* 152:667-672; Parronchi *et al.* (1997) *Am. J. Path.* 150:823-832). The invention provides methods for inhibiting IL-12 activity in a subject suffering from such a disorder, which method comprises administering to the subject an antibody or antibody portion of the invention such that IL-12 activity in the subject is inhibited.
35 Preferably, the IL-12 is human IL-12 and the subject is a human subject. Alternatively, the subject can be a mammal expressing a IL-12 with which an antibody of the invention cross-reacts. Still further the subject can be a mammal into which has been introduced hIL-12 (*e.g.*, by administration of hIL-12 or by expression of an hIL-12 transgene). An

antibody of the invention can be administered to a human subject for therapeutic purposes (discussed further below). Moreover, an antibody of the invention can be administered to a non-human mammal expressing a IL-12 with which the antibody cross-reacts for veterinary purposes or as an animal model of human disease. Regarding
5 the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (*e.g.*, testing of dosages and time courses of administration).

As used herein, the phrase "a disorder in which IL-12 activity is detrimental" is intended to include diseases and other disorders in which the presence of IL-12 in a subject suffering from the disorder has been shown to be or is suspected of being either
10 responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which IL-12 activity is detrimental is a disorder in which inhibition of IL-12 activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of IL-12 in a biological fluid of a subject
15 suffering from the disorder (*e.g.*, an increase in the concentration of IL-12 in serum, plasma, synovial fluid, *etc.* of the subject), which can be detected, for example, using an anti-IL-12 antibody as described above. There are numerous examples of disorders in which IL-12 activity is detrimental. In one embodiment, the antibodies or antigen binding portions thereof, can be used in therapy to treat the diseases or disorders
20 described herein. In another embodiment, the antibodies or antigen binding portions thereof, can be used for the manufacture of a medicine for treating the diseases or disorders described herein. The use of the antibodies and antibody portions of the invention in the treatment of a few non-limiting specific disorders is discussed further below:

25

A. Rheumatoid Arthritis:

Interleukin-12 has been implicated in playing a role in inflammatory diseases such as rheumatoid arthritis. Inducible IL-12p40 message has been detected in synovia from rheumatoid arthritis patients and IL-12 has been shown to be present in the
30 synovial fluids from patients with rheumatoid arthritis (see *e.g.*, Morita *et al.*, (1998) *Arthritis and Rheumatism* 41: 306-314). IL-12 positive cells have been found to be present in the sublining layer of the rheumatoid arthritis synovium. The human antibodies, and antibody portions of the invention can be used to treat, for example, rheumatoid arthritis, juvenile rheumatoid arthritis, Lyme arthritis, rheumatoid
35 spondylitis, osteoarthritis and gouty arthritis. Typically, the antibody, or antibody portion, is administered systemically, although for certain disorders, local administration of the antibody or antibody portion may be beneficial. An antibody, or antibody portion,

of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of autoimmune diseases.

In the collagen induced arthritis (CIA) murine model for rheumatoid arthritis, treatment of mice with an anti-IL-12 mAb (rat anti-mouse IL-12 monoclonal antibody, C17.15) prior to arthritis profoundly suppressed the onset, and reduced the incidence and severity of disease. Treatment with the anti-IL-12 mAb early after onset of arthritis reduced severity, but later treatment of the mice with the anti-IL-12 mAb after the onset of disease had minimal effect on disease severity.

10 B. Crohn's Disease

Interleukin-12 also plays a role in the inflammatory bowel disease, Crohn's disease. Increased expression of IFN- γ and IL-12 occurs in the intestinal mucosa of patients with Crohn's disease (see *e.g.*, Fais *et al.*, (1994) *J. Interferon Res.* 14: 235-238; Parronchi *et al.*, (1997) *Amer. J. Pathol.* 150: 823-832; Monteleone *et al.*, (1997) *Gastroenterology* 112: 1169-1178; Berrebi *et al.*, (1998) *Amer. J. Pathol.* 152: 667-672). Anti-IL-12 antibodies have been shown to suppress disease in mouse models of colitis, *e.g.*, TNBS induced colitis IL-2 knockout mice, and recently in IL-10 knockout mice. Accordingly, the antibodies, and antibody portions, of the invention, can be used in the treatment of inflammatory bowel diseases.

20 C. Multiple Sclerosis

Interleukin-12 has been implicated as a key mediator of multiple sclerosis. Expression of the inducible IL-12 p40 message or IL-12 itself can be demonstrated in lesions of patients with multiple sclerosis (Windhagen *et al.*, (1995) *J. Exp. Med.* 182: 1985-1996, Drulovic *et al.*, (1997) *J. Neurol. Sci.* 147: 145-150). Chronic progressive patients with multiple sclerosis have elevated circulating levels of IL-12. Investigations with T-cells and antigen presenting cells (APCs) from patients with multiple sclerosis revealed a self-perpetuating series of immune interactions as the basis of progressive multiple sclerosis leading to a Th1-type immune response. Increased secretion of IFN- γ from the T cells led to increased IL-12 production by APCs, which perpetuated the cycle leading to a chronic state of a Th1-type immune activation and disease (Balashov *et al.*, (1997) *Proc. Natl. Acad. Sci.* 94: 599-603). The role of IL-12 in multiple sclerosis has been investigated using mouse and rat experimental allergic encephalomyelitis (EAE) models of multiple sclerosis. In a relapsing-remitting EAE model of multiple sclerosis in mice, pretreatment with anti-IL-12 mAb delayed paralysis and reduced clinical scores. Treatment with anti-IL-12 mAb at the peak of paralysis or during the subsequent remission period reduced clinical

scores. Accordingly, the antibodies or antigen binding portions thereof of the invention may serve to alleviate symptoms associated with multiple sclerosis in humans.

D. Insulin-Dependent Diabetes Mellitus

5 Interleukin-12 has been implicated as an important mediator of insulin-dependent diabetes mellitus (IDDM). IDDM was induced in NOD mice by administration of IL-12, and anti-IL-12 antibodies were protective in an adoptive transfer model of IDDM. Early onset IDDM patients often experience a so-called "honeymoon period" during which some residual islet cell function is maintained. These
10 residual islet cells produce insulin and regulate blood glucose levels better than administered insulin. Treatment of these early onset patients with an anti-IL-12 antibody may prevent further destruction of islet cells, thereby maintaining an endogenous source of insulin.

15 *E. Psoriasis*

Interleukin-12 has been implicated as a key mediator in psoriasis. Psoriasis involves acute and chronic skin lesions that are associated with a TH1-type cytokine expression profile. (Hamid et al. (1996) J. Allergy Clin. Immunol. 1:225-231; Turka et al. (1995) Mol. Med. 1:690-699). IL-12 p35 and p40 mRNAs were detected in
20 diseased human skin samples. Accordingly, the antibodies or antigen binding portions thereof of the invention may serve to alleviate chronic skin disorders such psoriasis.

The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The contents of all cited
25 references, including literature references, issued patents, and published patent applications, as cited throughout this application are hereby expressly incorporated by reference. It should further be understood that the contents of all the tables attached hereto (see Appendix A) are incorporated by reference.

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420	2421	2422	2423	2424	2425	2426	2427	2428	2429	2430	2431	2432	2433	2434	2435	2436	2437	2438	2439	2440	2441	2442	2
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Table 10.2.3 VHB Family Germline Amino Acid Sequences

Numbering according to Kabat
(Joe9 VH included for comparison)

SEQ ID	germline	CDR H1		CDR H2	
		NO:	VH		
574	dp-29	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	HYMD	WVRQAPGKGLEWVG
575	DP-30	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	DHYMS	WVRQAPGKGLEWVG
576	HC15-7	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	DHYMS	WVRQAPGKGLEWVG
577	VHD26	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	DHYMS	WVRQAPGKGLEWVG
578	DP-31	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	DYAMH	WVRQAPGKGLEWVS
579	DP-32	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	DYAMH	WVRQAPGKGLEWVS
580	DP-33	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	DYAMH	WVRQAPGKGLEWVS
581	dp-35	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	DYMS	WVRQAPGKGLEWVS
582	VH3-8	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	DYMS	WVRQAPGKGLEWVS
583	yac-9	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	GSAMH	WVRQAPGKGLEWVS
584	dp-38	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NAWMS	WVRQAPGKGLEWVG
585	LSG2	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NAWMS	WVRQAPGKGLEWVG
586	LSG3	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NAWMS	WVRQAPGKGLEWVG
587	LSG4	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NAWMS	WVRQAPGKGLEWVG
588	LSG6	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NAWMS	WVRQAPGKGLEWVG
589	v3-15	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NAWMS	WVRQAPGKGLEWVG
590	dp-39	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NAWMS	WVRQAPGKGLEWVG
591	dp-40	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NAWMS	WVRQAPGKGLEWVG
592	dp-59	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
593	v3-16p	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
594	v3-19p	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
595	v3-13	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
596	DP-42	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
597	dp-44	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
598	DP-45	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
599	dp-47	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
600	flm	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
601	P1	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
602	v3-64	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
603	vh26	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
604	B25	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
605	b32e	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
606	B37	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
607	B43	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
608	B48	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
609	B52	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
610	B54	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
611	cos-8	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
612	dp-46	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
613	F2M	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
614	F3	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
615	F7	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
616	hv3005	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
617	P2	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
618	dp-48	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS
619	dp-58	EVQLVESGGGLVQP	GGSLRLSCAASGFTFS	NSDMN	WVRQAPGKGLEWVS

Table 1 VH3 Family Germline Amino Acid Sequences
Numbering according to Kabat
(Joe9 VH included for comparison)

SEQ ID	germline	CDR H1	CDR H2
640	VH		
641	B1	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
642	B18	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
643	B26	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
644	B28E	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
645	B29E	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
646	B29M	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
647	B30	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..WYDGSNKYYADSVKVG
648	B32M	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
649	cos-3	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
650	dp-49	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..WYDGSNKYYADSVKVG
651	dp-50	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..WYDGSNKYYADSVKVG
652	P6	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..WYDGSNKYYADSVKVG
653	P9E	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..WYDGSNKYYADSVKVG
654	v3-30	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
655	v3-33	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SYDGSNKYYADSVKVG
656	dp-51	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..WYDGSNKYYADSVKVG
657	dp-77	EVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
658	HG4	EVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
659	v3-21	EVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
660	v3-48	EVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
661	DP-52	EDQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
662	cos-6	EVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
663	dp-53	EVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
664	dp-54	EVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
665	dp-87	EVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
666	VH3-11	EVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA VI..SSSSSTIYYADSVKVG
667	JOE9 VH	QVQLVSGGGVVPQGRSLRLSCAASGFTFS	WVRQAPGKGLEWVA FI..RYDGSNKYYADSVKVG

Table 1 Vλ1 Family Germline Amino Acid Sequences
Numbering according to Kabat.
(Joe9 VL included for comparison)

SEQ ID	NO:	gene*	VL	CDR L1			CDR L2			CDR L3		
668	1b	DPL5	QSVLTQPPSVSAAPGQKVTISC	SGSSSNIGNNY.VS	WYQQLPGTAPKLLIY	DNNKRPS	GIPDRFSGSKSGTSATLGITGLQGEADYYC	GTWDSLSA				
669	1d	DPL4	QSVLTQPPSVSAAPGQKVTISC	SGSSDMGNYA.VS	WYQQLPGTAPKLLIY	ENNKRPS	GIPDRFSGSKSGTSATLGITGLWPDEADYYC	LAWDTSRA				
670	1c	DPL2	QSVLTQPPSVASGTPGQRVTISC	SGSSSNIGSNT.VN	WYQQLPGTAPKLLIY	SNNQRPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AWDDSLNG				
671	1g	DPL3	QSVLTQPPSVASGTPGQRVTISC	SGSSSNIGSNT.VY	WYQQLPGTAPKLLIY	RNNQRPS	GVPDRFSGSKSGTSASLAISGLRSEDEADYYC	AWDDSLSG				
672	1a	DPL1	QSVLTQPPSVSEAPRQRTISC	SGSSSNIGNN.AVN	WYQQLPGTAPKLLIY	YDDLPS	GVPDRFSGSKSGTSASLAISGLQSEDEADYYC	AWDDSLNG				
673	1f	DPL9	QSVLTQPPSVSEAPRQRTISC	TGSSSNIGAGYVH	WYQQLPGTAPKLLIY	GNSNRPS	GVPDQFSGSKSGTSASLAITGLQSEDEADYYC	KAWDNLNA				
674	1e	DPL8	QSVVTQPPSVSEAPGQRVTISC	TGSSSNIGAGYDVH	WYQQLPGTAPKLLIY	GNSNRPS	GVPDRFSGSKSGTSASLAITGLQAEDEADYYC	QSYDSSLG				
675	JOE9 VL		SVTLTQPPSVSGTTPGQRVTISC	SGCRSNIGSNT.VK	WYQQLPGTAPKLLIY	GNDQRPS	GVPDRFSGSKSGTSASLAITGVQAEDEADYYC	QSYDSSLRG				

*Williams, JMB, 1996, 264, 220-232

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
Joe9 wt	77	SGSYD	110	QSYDSSLRGSRV	1.00E-01	1.50E-06	1.00E-06	
Joe9 wt IgG1	77	SGSYD	110	QSYDSSLRGSRV			5.00E-07	
70-1	78	HGSHDN	110	Joe9 wt	1.34 e-2		2.00E-07	
70-1 IgG1	78	HGSHDN	110	Joe9 wt			2.00E-07	
70-2	79	HGSHDN	110	Joe9 wt	3.30E-02		3-5.0E-7	
70-7	80	RRRSNY	110	Joe9 wt	1.29E-01		3-5.0E-7	
70-13	81	SGSIDY	110	Joe9 wt	7.20E-02		3-5.0E-7	
78-34	77	wt	111	QSYDRGFTGSRV	1.64 e-2	2.00E-07	6.00E-07	
78-25	77	wt	112	QSYDSSLRGSRV	5.00E-02			
78-28	77	wt	112	QSYDSSLRGSRV	4.66E-02			
78-35	77	wt	113	QSYDSSLTGSRV	4.99E-02	4.00E-07		
79-1	77	wt	114	QSYDSSLWGSRV		2.00E-07	6.00E-07	
101-14	79	70-2	111	78-34	7.52E-03			
101-9	79	70-2	113	78-35	8.54E-03			
101-19	81	70-13	111	78-34	4.56E-02			
101-8	81	70-13	111	78-34	1.01E-02			
101-4	81	70-13	113	78-35	9.76E-03			
101-5	81	70-13	113	78-35	4.45E-02			
101-11 (12)	78	70-1	111	78-34	4.5 e-3		3.00E-08	
101-11 IgG1	78	70-1	111	78-34		1.60E-09		
26-1 (2,3)	78	70-1	114	79-1	7.4 e-3		6.00E-08	
136-9	82	HGSHDD	115	QTYDISESGSRV	3.20E-03			
136-10	82	HGSHDD	116	QSYDRGFTGSRV	1.40E-03	2.00E-09		
136-14	83	HGSHDN	117	QTYDRGFTGSRV	1.10E-03	3.00E-10	1.00E-07	
136-15	83	HGSHDN	118	QTYDKGFTGSSV	7.4 e-4	1.00E-10	2.00E-09	
136-15 germline	83	HGSHDN	118	QTYDKGFTGSSV	4.60E-04		6.00E-09	
136-16	83	HGSHDN	119	QSYDRRFTGSRV	6.10E-04	3.00E-10	5.00E-09	
136-17	83	HGSHDN	120	QSYDWNFTGSRV	2.90E-05	2.00E-09	7.00E-09	
136-18	83	HGSHDN	121	QSYDRGFTGSRV	1.10E-03	8.00E-10		
136-21	83	HGSHDN	122	QSYDNGFTGSRV	4.20E-04	2.00E-09		
136-24	83	HGSHDN	123	QSYDNAVTASKV	8.90E-04	1.00E-09		

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
101-11	84	TT HGSHDN WGQG	124	QSYDRGFTGSRV	4.5x10 ⁻³	2x10 ⁻⁹	2.00E-08	
136-15M1	85	AK	124	QSYDRGFTGSRV		4.00E-10		
149-4	86S..	124	1.37x10 ⁻³	8x10 ⁻¹¹	3.00E-09	
149-5	87T	125	QSYDSSLWGTRV	1.02x10 ⁻³	1.2x10 ⁻¹⁰	3.00E-09	
149-6	84S..	124	2.73x10 ⁻³	6x10 ⁻¹⁰	2.00E-09	
149-7	84S..	126D.....	1.13x10 ⁻³	9x10 ⁻¹⁰	3.00E-09	
149-8	88	K.			2.33x10 ⁻³	3x10 ⁻⁹		
149-9	89	K.H.	127	...E.....M.	3.54x10 ⁻³	1.8x10 ⁻¹⁰		
149-11	90S..	128N....A..	1.43x10 ⁻²	2x10 ⁻¹⁰	4.00E-09	
149-12	84S..			3.73x10 ⁻³	neutralising		
149-13	84S..			2.22x10 ⁻³	5x10 ⁻¹⁰		
149-14	91	.. .R..N.				1.5x10 ⁻¹⁰	6.00E-09	
156-1	92	TT HGSHDN	124	QSYDRGFTGSRV				
156-2	93T	126D.....	5.00E-03			
156-3	93T	129R.....				
156-4	93T	128N....A..	9.00E-03			
156-5	93T	127	...E.....SM.				
156-6	92T	130	.T..K.....S.				
156-7	92S..	126D.....	3.00E-03			
156-8	92S..	129R.....				
156-9	92S..	128N....A..				
156-10	92S..	127	...E.....SM.				
156-11	94	.K	130	.T..K.....S.				
156-12	94	.K	126D.....				
156-13	94	.K	129R.....				
156-14	94	.K	128N....A..				
156-15	94	.K	127	...E.....SM.				
156-16	93T	130	.T..K.....S.				
156-17	92S..	124				
156-18	93T	125SSLW.T..	6.00E-03			
			125SSLW.T..				

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
	92	TT HGSHDN	124	QSYDRGFTGSRV				
103-1	95	.. Q.R...	124	2.9x10 ⁻³			
103-2	96	K. R.R...	130	.T..K.....S.	7.3x10 ⁻⁴	7.00E-11	1.00E-09	
103-3	97K	124	2.5x10 ⁻³			
103-6			131D...T..	4.5x10 ⁻⁴			
103-7	98D	131D...T..	3.7x10 ⁻⁴	1.40E-10	1.00E-09	
103-8	99	K.	130	.T..K.....S.	3.3x10 ⁻⁴	6.00E-11	1.50E-09	
103-14 & 9	100	KT HGSHDN	132	QSYDRGFTGSMV	6.7 e-4	4.00E-11	1.20E-09	
103-8 & 2	100	KT HGSHDN	133	QTYDKGFTGSSV	5.3 e-4		1.50E-09	
103-4	101	TT HGSHDN	134	QSYDRGFTGARV	1.6 e-4	8.60E-11	9.00E-10	
103-152	101	TT HGSHDN	135	QSYERGTGARV		8.60E-11		
	102	TT SSSYDY	136	QSYDRGFTGSRVF				
170-1	102	137FK..	2.35E-03			
170-2	102	138VSAY..	8.80E-04			
170-3	102	139L.VTK..	1.11E-03			
170-4	102	140Y.A....	8.11E-04			
170-7	102	141K..	5.30E-04			
170-11	102	142L..F...	4.40E-04			
170-13	102	143YK..	1.59E-03			
170-15	102	144L..Y.L.	4.43E-03			
170-19	103	.. H..H.N	145DYK..	1.00E-03			
170-21	104	.. H..Q.N	146P.L.	3.89E-03			
170-22	102	147L.....	5.60E-04			
170-23	103	.. H..H.N	148A..W	1.00E-03	2.00E-10		
170-24	104	.. H..Q.N	149Y...	2.80E-04	5.00E-10		
170-35	105	A. H..Q.N	136	1.00E-05			
170-38			150P...	2.10E-04			
170-39			151M.S....	2.79E-03			
170-36	83	HGSHDN	152	QSYDRDSTGSRVF	4.00E-04	2.00E-10		
170-25	106	HGSQDT	153	QSYDSSLRGSRVF	5.00E-04	5.00E-11		

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
	106	SGSYDY	136	QSYDGRGTGSRVFE				
73-B1	107	SGSYDY	154	H...SD.....	3.25E-03	>1E-8		
73-B2	107	SGSYDY	155	H.SES.....	2.07E-03			
73-B6	107	SGSYDY	156	H...NR.....	2.51E-03	>1E-8		
73-C1	107	SGSYDY	157	H...SR.....	2.71E-03	>1E-8		
73-C2	107	SGSYDY	158	...SE.....	3.79E-03			
73-C6	107	SGSYDY	159	...T.....	3.96E-03			
73-D1	107	SGSYDY	160	H...S.....	3.99E-03			
73-D2	107	SGSYDY	161	...T.....	3.56E-03			
73-D4	107	SGSYDY	162	H...TK.....	5.36E-03			
73-D5	107	SGSYDY	163	H.S.S.....	3.57E-03			
73-E3	107	SGSYDY	164	...SD.....	4.98E-03			
73-E6	107	SGSYDY	165	H..ES.....	4.17E-03			
73-F3	107	SGSYDY	166	...APWS.....	7.08E-03			
73-F5	107	SGSYDY	167	...DSD....K..	3.74E-03			
73-G2	107	SGSYDY	168	HTN.S.....	3.98E-03			
73-G3	107	SGSYDY	169	H...TR.....	3.50E-03			
73-G4	107	SGSYDY	170	...MR.....	6.58E-03			
73-G5	107	SGSYDY	171	H.S.SDS.....	6.01E-03			
73-G6	107	SGSYDY	172	...NTD.....	6.30E-03			
73-H2	107	SGSYDY	173	...S.....	5.93E-03			
73-F6	107	SGSYDY	174	H...M.....	5.87E-03			
73-H3	107	SGSYDY	175	H...N.....	6.85E-03			
73-C5	107	SGSYDY	176	H.H..D.....	4.84E-03			
73-B7	108	HGSQDN	177	QSYDSSLRGSRV	2.50E-03	7.00E-09		

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
			136	QSYDRGFTGSRVF				
M2 A2	83	HGSHDN	178IH.....	4.00E-02			
M2 A4	83	HGSHDN	179S..P.....	8.49E-03			
M2 A5	83	HGSHDN	180I.S.....	4.01E-02			
M2 B1	83	HGSHDN	181S.L.....	7.97E-03			
M2 B3	83	HGSHDN	182I.M.....	4.60E-02			
M2 B4	83	HGSHDN	183I.L.....	4.42E-02			
M2 B5	83	HGSHDN	184S.V.....	8.38E-03			
M2 B6	83	HGSHDN	185L.A.....	2.81E-02			
M2 C2	83	HGSHDN	181S.L.....	4.85E-02			
M2 C3	83	HGSHDN	186T.L.....	4.62E-02			
M2 C4	83	HGSHDN	181S.L.....	8.16E-03			
M2 C5	83	HGSHDN	187TAL.....	4.71E-02			
M2 D1	83	HGSHDN	188IR.....	3.71E-02			
M2 D2	83	HGSHDN	189IRS.....	3.85E-02			
M2 D3	83	HGSHDN	190NRL.....	3.33E-02			
M2 D4	83	HGSHDN	191ETS.....	5.81E-02			
M2 D5	83	HGSHDN	192SSS.....	5.18E-02			
M2 D6	83	HGSHDN	193S...A....	5.01E-02			
M2 E1	83	HGSHDN	194	.T..K.....S..	5.32E-02			
M2 E2	83	HGSHDN	195N.....	4.77E-02			
M2 E6	83	HGSHDN	196T...K....	9.77E-03			
M2 F1	83	HGSHDN	197SDV.....	6.16E-02			
M2 H5	83	HGSHDN	198A.....	9.90E-03			

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
			124	OSYDRGFTGSRV				
A5	83	HGSHDN	199THPSML	1.12E-03			
A12	83	HGSHDN	200TTPRPM	1.43E-03			
A4	83	HGSHDN	201RNPALT	1.47E-03			
A6	83	HGSHDN	202THPWLH	1.87E-03			
A10	83	HGSHDN	203NSPATV	1.87E-03			
A11	83	HGSHDN	204TFPSPQ	2.07E-03			
C2	83	HGSHDN	205LNFSAF	2.23E-03			
A8	83	HGSHDN	206KSNKML	2.37E-03			
B8	83	HGSHDN	207HTAHLY	2.40E-03			
C6	83	HGSHDN	208QTPSIT	2.42E-03			
A3	83	HGSHDN	209YPRNIL	2.51E-03			
B11	83	HGSHDN	210ITPGLA	2.95E-03			
B5	83	HGSHDN	211QPHAVL	3.04E-03			
C10	83	HGSHDN	212NSPIPT	3.10E-03			
C4	83	HGSHDN	213TPNNSF	3.23E-03			
C3	83	HGSHDN	214S.VDPGPY	3.34E-03			
B2	83	HGSHDN	215RPRHAL	3.61E-03			
A2	83	HGSHDN	216PYHPIR	3.80E-03			
C5	83	HGSHDN	217PHTQPT	3.91E-03			
A7	83	HGSHDN	218HNNFSP	3.95E-03			
C9	83	HGSHDN	219PHTLPH	3.97E-03			
B3	83	HGSHDN	220TPSYPT	4.12E-03			
C8	83	HGSHDN	221S.TSNLLP	5.36E-03			
B7	83	HGSHDN	222DSNHDL	5.45E-03			
A1	83	HGSHDN	223LPRLTH	5.66E-03			
C7	83	HGSHDN	224IPTSYL	5.83E-03			
C12	83	HGSHDN	225LRVQAP	5.85E-03			
B10	83	HGSHDN	226LSDSPL	6.04E-03			
B6	83	HGSHDN	227S.SLRRIL	7.58E-03			
A9	83	HGSHDN	228PARTSP	7.98E-03			
B9	83	HGSHDN	229RAHPQ	8.66E-03			

Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
			124	QSYDRGFTGSRV				
177-D7	83	HGSHDN	230TOPABI	4.07E-04			
177-G6	83	HGSHDN	231THPTMI	5.50E-04			
177-D9	83	HGSHDN	232RIPABT	6.32E-04			
177-C6	83	HGSHDN	233THPVPA	7.94E-04			
177-H5	83	HGSHDN	234SBPIPA	1.32E-03			
177-H9	83	HGSHDN	235THPVPA	1.58E-03			
177-H10	83	HGSHDN	236THPTMY	3.44E-03			
144-F1	83	HGSHDN	237HHYTF	5.80E-04			
43-E3	83	HGSHDN	238SHPAAE	8.00E-04			
43-E9	83	HGSHDN	239TIPSIE	8.00E-04			
43-G2	83	HGSHDN	240SSPAIM	7.00E-04			
43-G3	83	HGSHDN	241IWPNLN	9.00E-04			
31-A6	83	HGSHDN	242THPNLN	5.00E-04			
31-B5	83	HGSHDN	243THPSIS	5.00E-04			
			124	QSYDRGFTGSRV				
Y17	83	HGSHDN	244	QSYDRGSAPMIN	8.90E-05	4.50E-10	>1E-8	
Y19	83	HGSHDN	245	QSYDRGHPAMS	2.26E-04	3.00E-11	>1E-8	
Y38	83	HGSHDN	246THPSIT	5.08E-04	5.50E-11	2.60E-09	
Y45	83	HGSHDN	247TDPaIV	6.17E-04	4.00E-11	4.30E-09	
Y61	83	HGSHDN	248THPALL	2.75 e-4	4E-11	1.40E-10	
Y61 IgG	83	HGSHDN	248THPALL	1.50E-04	1.60E-11	1.30E-10	
Y61 IgG germline	83	HGSHDN	248THPALL	1.50E-04	1.60E-11	1.30E-10	1.60E-10
Y139	83	HGSHDN	249SHPALT	5.92E-04	3E-11	4.50E-10	
Y139 IgG1	83	HGSHDN	249SHPALT			1.00E-09	
Y174	83	HGSHDN	250TTPAPE	7.55E-04	6E-11	2.00E-09	
Y177	83	HGSHDN	251SHPTLI	6.61E-04	5E-11	1.00E-09	
A5	83	HGSHDN	252THPSML	4.50E-04	6.60E-11		
A12	83	HGSHDN	253TTPRPM	5.57E-04	2.50E-10		
D9	83	HGSHDN	254RLPAQT	8.21E-04	3.5E-09	>>	
G6	83	HGSHDN	255THPLTI	5.08E-04	1E-10	1.00E-09	
G6 IgG1	83	HGSHDN	255THPLTI			1.00E-09	
C6	83	HGSHDN	256	QSYDRGQTPSIT	1.07E-03	3.5E-10	1.00E-08	
Y55	83	HGSHDN	257	QSYDRGTHFQMY	1.06E-03	1.40E-10	>1E-8	

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
A4	83	HGSHDN	258	QSYDRGNPALT	6.30E-04	2.50E-10		
AO3	83	HGSHDN	259	QSYDRGTHPLTM	3.04E-04	3.00E-11	4.00E-10	
AO3 IgG1	83	HGSHDN	260	QSYDRGTHPLTM	3.04 e-4	2.90E-11	3.80E-10	
AO3 IgG germline	83	HGSHDN	260	QSYDRGTHPLTM	2.50E-04	3.50E-11	1.75E-10	
99-B11	83	HGSHDN	261	QSYDSGYTGSRV	5.40E-03			
99-C11	83	HGSHDN	262	QSYDSGFTGSRV	5.70E-03			
99-H4	83	HGSHDN	263	QSYDSRFTGSRV	4.80E-03			
99-E9	83	HGSHDN	262	QSYDSGFTGSRV	5.40E-03			
99-H7	83	HGSHDN	264	QSYPDGTPASRV	3.30E-03			
99-H11	83	HGSHDN	265	QSYSTHMPISRV	4.90E-03			
99-F6	83	HGSHDN	266	QSYDSGSTGSRV	4.90E-03			
99-F7	83	HGSHDN	267	QSYNSYPISRV	4.80E-03			
99-F8	83	HGSHDN	268	QSYRAPQOV	3.70E-03			
99-F11	83	HGSHDN	262	QSYDSGFTGSRV	5.40E-03			
99-G7	83	HGSHDN	269	QSYLKSRAFSRV	4.80E-03			
99-G11	83	HGSHDN	270	QSYDSRFTGSRV	4.30E-03			
L3.3R3M-B1	83	HGSHDN	124	QSYDRGFTGSRV				
L3.3R3M-B3	83	HGSHDN	271FTGSMV	5.46E+00			
L3.3R3M-C6	83	HGSHDN	272FTGSMV	5.51E+00			
L3.3R3M-F9	83	HGSHDN	273FTGFDG	6.17E+00			
L3.3R3M-G8	83	HGSHDN	274TAPALS	4.99E+00			
L3.3R3M-H6	83	HGSHDN	275SYPALR	5.55E+00			
L3.3R3M-H10	83	HGSHDN	276NWPNSN	5.69E+00			
L3.3R3M-A3	83	HGSHDN	277TAPSLI	5.35E+00			
L3.3R3M-F8	83	HGSHDN	278FTGSMV	5.37E+00			
L3.3R3M-G1	83	HGSHDN	279TTPRIR	4.99E+00			
L3.3R3M-G7	83	HGSHDN	280FTGSMV	4.21E+00			
L3.3R3M-H11	83	HGSHDN	281FTGSMV	4.24E+00			
	83	HGSHDN	282MIPALT	3.95E+00			

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
Y61-L94N	109	CKT HGSHDN	283	QSYDRNTHPALL			8.00E-11	
Y61-L94F	109	CKT HGSHDN	284	QSYDRFTHPALL			6.00E-11	
Y61-L94Y	109	CKT HGSHDN	285	QSYDRYTHPALL		2.00E-11	2.00E-11	
Y61-L94Y IgG	109	CKT HGSHDN	285	QSYDRYTHPALL	1.27E-04	6.00E-11	5.00E-11	4.00E-11
Y61-L50Y	109	CKT HGSHDN	286	QSYDRGTHPALL		2.00E-11		2.00E-11
Y61-L50Y* IgG	109	CKT HGSHDN	286	QSYDRGTHPALL	6.98E-05		2.00E-11	3.00E-11
Y61-L50Y-H31E** IgG	109	CKT HGSHDN	286	QSYDRGTHPALL	2.99E-05		6.00E-11	2.00E-11
Y61-L50Y-H31E- L94Y** IgG	109	CKT HGSHDN	287	QSYDRYTHPALL	4.64E-05		1.00E-11	1.00E-11
J695 (Y61-L94Y- L50Y IgG*)	109	CKT HGSHDN	287	QSYDRYTHPALL	5.14E-05	5.00E-11	1.00E-11	5.00E-12

*CDR L2: L50G to Y

**CDR L2: L50G to Y; CDR H1: H31S to E

Table 3									
Kabat Number	CDR H1				CDR H2				CDR H3
	27	28	29	30	31	32	33	34	35
Y61 VH	F	T	F	S	S	Y	G	M	H
Contact Positions				X	X	X	X	X	X
Hypermutation Positions				X	X	X			
Kabat number	CDR L1				CDR L2				CDR L3
	24	25	26	27	27A	27B	28	29	30
Y61 VL	S	G	G	R	S	N	I	G	S
Contact Positions									
Hypermutation Positions									
Kabat number	CDR L1				CDR L2				CDR L3
	97	95C	95B	95A	95	94	93	92	91
Y61 VL	S	Q	S	Y	D	R	G	T	H
Contact Positions									
Hypermutation Positions									
x contact and/or hypermutation position x contact and/or hypermutation position mutated in Y61									

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Table 4. Neutralization Activity in the Presence of Excess Free IL-12 p40					
SEQ ID NO:	Clone	PHA assay IC50 (M) p70:p40 1:0	PHA assay IC50 (M) p70:p40 1:20	PHA assay IC50 (M) p70:p40 1:50	
VH: 47	136-15	2.00E-09	5.00E-09	4.00E-09	
VL: 48					
VH: 51	149-5	6.50E-09	7.00E-09	4.00E-09	
VL: 52					
VH: 53	149-6	9.00E-10	1.00E-09	1.00E-09	
VL: 54					
VH: 84	149-7	3.50E-09	2.50E-09	4.00E-09	
VL: 126					
VH: 23	Y61 IgG	1.80E-10		1.80E-10	
VL: 24					
VH: 65	AO3 IgG1	2.50E-10		2.20E-10	
VL: 66					
VH: 31	J695	1.00E-11		3.50E-11	
VL: 32					

EXAMPLES

EXAMPLE 1: Isolation of Anti-IL-12 Antibodies

5 A. Screening for IL-12 binding antibodies

Antibodies to hIL-12 were isolated by screening three separate scFv phage display libraries prepared using human VL and VH cDNAs from mRNA derived from human tonsils (referred to as scFv 1), tonsil and peripheral blood lymphocytes (PBL) (referred to as scFv 2), and bone marrow-derived lymphocytes (referred to as
10 BMDL). Construction of the library and methods for selection are described in Vaughan et al. (1996) Nature Biotech. 14: 309-314.

The libraries were screened using the antigens, human IL-12 p70 subunit, human IL-12 p40 subunit, chimaeric IL-12 (mouse p40/human p35), mouse IL-12, biotinylated human IL-12 and biotinylated chimaeric IL-12. IL-12 specific antibodies
15 were selected by coating the antigen onto immunotubes using standard procedures (Marks et al., (1991) J. Mol. Biol. 222: 581-597). The scFv library 2 was screened using either IL-12, or biotinylated-IL-12, and generated a significant number of IL-12 specific binders. Five different clonotypes were selected, determined by BstN1 enzymatic digestion patterns, and confirmed by DNA sequencing. The main clonotypes were
20 VHDP58/VLDPL11, VHDP77/VLDPK31, VHDP47/VL and VHDP77/VLDPK31, all of which recognized the p40 subunit of IL-12.

Screening of the BMDL library with IL-12 p70 generated 3 different clonotypes. Two of these were found to be cross-reactive clones. The dominant clone was sequenced and consisted of VHDP35/VLDP. This clone recognizes the p40 subunit of
25 IL-12. Screening of the scFv library 1, using IL-12 p70, did not produce specific IL-12 antibodies.

In order to identify IL-12 antibodies which preferentially bind to the p70 heterodimer or the p35 subunit of IL-12, rather than the p40 subunit, the combined scFv 1 + 2 library, and the BMDL library were used. To select IL-12 antibodies that
30 recognized the p70 heterodimer or p35 subunit, phage libraries were preincubated and selected in the presence of free p40. Sequencing of isolated clones revealed 9 different antibody lineages. Subunit preferences were further analyzed by 'micro-Friguet' titration. The supernatant containing scFv was titrated on biotin-captured IL-12 in an ELISA and the ED₅₀ determined. The concentration of scFv producing 50% ED was
35 preincubated with increasing concentrations of free p70 or p40 (inhibitors). A decrease in the ELISA signal on biotin-IL-12 coated plates was measured and plotted against the concentration of free p70 or p40. This provided the IC₅₀ for each clone with respect to

p70 and p40. If the titrations for both subunits overlaps, then the scFv binds to both p40 and p70. Any variation from this gives the degree of preference of p70 over p40.

B. Affinity Maturation of Antibody Lineage Specific for IL-12 (Joe 9)

5 The clones were tested for their ability to inhibit IL-12 binding to its receptor in an IL-12 receptor binding assay (referred to as RBA), and for their ability to inhibit IL-12 induced proliferation of PHA stimulated human blast cells (PHA assay), described in Example 3. Clone Joe 9 had the lowest IC₅₀ value in both the RBA and the PHA assay, with an IC₅₀ value of 1 x 10⁻⁶ M in both assays. In addition the heavy chain variable
10 region (VH) of Joe 9 had the least number of changes compared to the closest germline sequence COS-3, identified from the VBASE database. Table 1 (see Appendix A) shows the V_H3 family of germline sequences, of which COS-3 is a member, as well as members of V_λ1 family of germline sequences. Therefore, Joe 9 was selected for affinity maturation. The amino acids sequences of VH and VL of the Joe9 wild type
15 (Joe9 wt) antibody are shown in Figure 1A-1D.

In order to increase the affinity of Joe 9, various mutations of the complementarity determining region 3 (CDR3) of both the heavy and light chains were made. The CDR3 variants were created by site-directed PCR mutagenesis using degenerate oligonucleotides specific for either the heavy chain CDR3 (referred to as
20 "H3") or the light chain CDR3 (referred to as "L3"), with an average of three base substitutions in each CDR3 (referred to as "spike"). PCR mutagenesis of the heavy chain CDR3 was performed using the degenerate heavy chain oligonucleotide containing a random mixture of all four nucleotides,
5'TGTCCCTTGGCCCCA(G)(T)(A)(G)(T)(C)(A)(T)(A)(G)(C)(T)(C)(C)(A)(C)(T)
25 GGTCGTACAGTAATA 3' (SEQ ID NO: 580), and oligonucleotide pUC Reverse Tag GAC ACC TCG ATC AGC GGA TAA CAA TTTCAC ACA GG (SEQ ID NO: 581) to generate a repertoire of heavy chain CDR3 mutants. The parent light chain was amplified using Joe 9 reverse oligonucleotide (5'TGG GGC CAA GGG ACA3' (SEQ ID NO:582) and the fdteteseq 24+21 oligonucleotide (5'-ATT CGT CCT ATA CCG TTC
30 TAC TTT GTC GTC TTT CCA GAC GTT AGT-3' (SEQ ID NO: 583).

Complementarity between the two PCR products was used to drive annealing of the two fragments in a PCR assembly reaction and the full length recombined scFv library was amplified with pUC Reverse Tag (SEQ ID NO: 581) and fdTag 5'-ATT CGT CCT ATA CCG TTC-3' (SEQ ID NO: 584). PCR mutagenesis of the light chain was
35 performed using the light chain oligonucleotide containing a mixture of all four nucleotides

5'GGTCCCAGTTCCGAAGACCCTCGAACC(C)(C)(T)(C)(A)(G)(G)(C)(T)
 (G)(C)(T)(G)(T)(C)ATATGACTGGCAGTAATAGTCAGC 3' (SEQ ID NO: 585), and
 Joe 9 reverse oligonucleotide 5'TGG GGC CAA GGG ACA3' (SEQ ID NO: 586)
 to produce a repertoire of light chain CDR3 mutants. The parent heavy chain was
 5 amplified with pUC Reverse Tag (SEQ ID NO: 581) and HuJH3FOR oligonucleotide
 5'TGAAGAGACGGTGACCATTGTCCC3' (SEQ ID NO: 587). Complementarity
 between the two PCR products was used to drive annealing of the two fragments in a
 PCR assembly reaction and the full length recombined scFv library was amplified with
 Reverse Tag GAC ACC TCG ATC AGC G (SEQ ID NO: 588) and HuJλ 2-3 FOR
 10 NOT oligonucleotide 5'GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC
 GGT CAG CTT GGT CCC 3' (SEQ ID NO: 589).

Heavy chain CDR3 mutants were selected using 1 nM biotinylated IL-12, and
 washed for 1 h at room temperature in PBS containing free IL-12 or p40 at a
 concentration of 7 nM. Clones were analyzed by phage ELISA and those that bound to
 15 IL-12 were tested in BIAcore kinetic binding studies using a low density IL-12 chip (see
 procedure for BIAcore analysis in Example 5). Generally, BIAcore analysis measures
 real-time binding interactions between ligand (recombinant human IL-12 immobilized
 on a biosensor matrix) and analyte (antibodies in solution) by surface plasmon resonance
 (SPR) using the BIAcore system (Pharmacia Biosensor, Piscataway, NJ). The system
 20 utilizes the optical properties of SPR to detect alterations in protein concentrations
 within a dextran biosensor matrix. Proteins are covalently bound to the dextran matrix
 at known concentrations. Antibodies are injected through the dextran matrix and
 specific binding between injected antibodies and immobilized ligand results in an
 increased matrix protein concentration and resultant change in the SPR signal. These
 25 changes in SPR signal are recorded as resonance units (RU) and are displayed with
 respect to time along the y-axis of a sensorgram. To determine the off rate (k_{off}), on rate
 (k_{on}), association rate (K_a) and dissociation rate (K_d) constants, BIAcore kinetic
 evaluation software (version 2.1) was used. Clones that demonstrated an improvement
 in the k_{off} rate were analyzed by neutralization assays which included inhibition by
 30 antibody of IL-12 binding to its receptor (RBA assay), inhibition of IL-12-induced
 proliferation in PHA stimulated human blast cells (PHA assay), and inhibition of IL-12-
 induced interferon gamma production by human blast cells (IFN gamma assay). A
 summary of the dissociation rates and/or IC_{50} values from neutralization assays of heavy
 chain CDR3 spiked clones 70-1 through 70-13 is presented in Table 2 (see Appendix A).
 35 Clone 70-1 displayed a k_{off} rate that was better than the parent Joe 9 clone, and had the
 lowest IC_{50} value of 2.0×10^{-7} M. Therefore clone 70-1 was selected for conversion to
 complete IgG1.

Light chain CDR3 mutants were selected using 1 nM biotin-IL-12 and washed with PBS containing 7 nM free p40. Clones were screened in phage ELISA and those that bound to IL-12 were tested in BIAcore binding analysis using low density IL-12 chips. Clones that displayed an off rate which was better than the parent Joe 9 clone
 5 were tested in neutralization assays which measured either, inhibition of IL-12 receptor binding, or inhibition of PHA blast cell proliferation. A summary of the dissociation rates and/or IC₅₀ values from neutralization assays of light chain CDR3 mutant clones, 78-34 through 79-1, is presented in Table 2 (see Appendix A).

Based on the k_{off} rate, clones 78-34 and 78-35 displayed an improved k_{off} rate
 10 compared to the parent Joe 9. Both of these clones were selected for combination analysis with heavy chain mutants.

C. Combination Clones

Mutant light and heavy chain clones that exhibited the best binding
 15 characteristics were used for combination and assembly of scFvs. Mutant clones with improved potency characteristics were combined by PCR overlap extension and pull-through of the mutated VH and VL segments as described above. Clones 101-14 through 26-1, shown in Table 2 (see Appendix A), were produced from the combination of heavy chain mutants (70-2, 70-13 and 70-1) with light chain mutants (78-34, 78-35
 20 and 79-1). The k_{off} rates and/or IC₅₀ values from neutralization assays for these clones are presented in Table 2.

BIAcore binding analysis identified clone 101-11, produced from the combination of the heavy chain CDR3 mutant clone 70-1 with the light chain CDR3 mutant clone 78-34, as having an off rate of 0.0045 s⁻¹. This k_{off} rate was a significant
 25 improvement compared to the k_{off} rates for either the heavy chain CDR3 mutant clone 70-1 (0.0134 s⁻¹), or for the light chain CDR3 mutant clone 78-34 (0.0164 s⁻¹) alone. Furthermore, clone 101-11 showed a significant improvement in neutralization assays. Accordingly, clone 101-11 was selected for affinity maturation as described below.

30 D. Affinity maturation of clone 101-11

Further affinity maturation of clone 101-11 consisted of repeat cycles of PCR mutagenesis of both the heavy and light chain CDR3s of 101-11 using spiked oligonucleotide primers. The clones were selected with decreasing concentrations of biotinylated IL-12 (bio-IL-12). The binding characteristics of the mutated clones was
 35 assessed by BIAcore binding analysis and RBA, PHA neutralization assays. The k_{off} rates and/or IC₅₀ values for clones 136-9 through 170-25 are presented in Table 2 (see Appendix A). Clone 103-14 demonstrated an improved IC₅₀ value in both the receptor

binding assay and the PHA blast assay. Clone 103-14 also demonstrated a low k_{off} rate, and accordingly was selected for further affinity maturation.

E. Generation and Selection of Randomized Libraries of Clone 103-14 Light CDR3

5 The light chain CDR3 of clone 103-14 (QSYDRGFTGSMV (SEQ ID NO: 590)) was systematically randomized in 3 segments using 3 different libraries as outlined below, where X is encoded by a randomized codon of sequence NNS with N being any nucleotide and S being either deoxycytosine or deoxyguanine.

L3.1=XXXXXXFTGSMV (SEQ ID NO: 591)

10 L3.2= QSYXXXXXXSMV (SEQ ID NO: 592)

L3.3=QSYDRGXXXXXX (SEQ ID NO: 593)

Randomized mutagenesis of all three light chain CDRs (referred to as L3.1, L3.2, and L3.3) of clone 103-14 was performed. The heavy chain CDR3 (referred to as H3) of clone 103-14 was not mutated. Four randomized libraries based on clone 103-14 (H3 and L3.1, L3.2 & L3.3) were constructed and subjected to a large variety of selection conditions that involved using limiting antigen concentration and the presence or absence of excess free antigen (p40 and p70). The outputs from selections (clones 73-B1 through 99-G11) were screened primarily by BIAcore, and on occasion with RBA and are shown in Table 2 (see Appendix A).

20 Random mutagenesis of the light chain CDR of 103-14 generated clone Y61, which exhibited a significant improvement in IC_{50} value compared to the parent clone 103-14. Y61 was selected for conversion to a whole IgG1. Whole Y61-IgG1 has an IC_{50} value of approximately 130 pM determined by the PHA assay. The IC_{50} value was not affected by a 50 fold molar excess of free p40, demonstrating that free p40 did not cross-react with Y61 anti-IL-12 antibody to thereby decrease the antibody binding to the heterodimer. The full length sequences of Y61 heavy chain variable region and light chain variable region are shown below.

30 Y61 Heavy Chain Variable Region Peptide Sequence

CDR H1

QVQLVESGGGVVQPGRSLRLSCAASFTFS **SYGMH** WVRQAPGKGLEWVA

35 **CDR H2**

FIRYDGSNKYYADSVKG RFTISRDN SKNTLYLQMNSLRAEDTAVYYCKT

CDR H3

HGSHDN WGQGTMTVTVSS (SEQ ID NO: 23)

40

Y61 Light Chain Variable Region Peptide Sequence

CDR L1

QSVLTQPPSVSGAPGQRTISC **SGGRSNIGSNTVK** WYQQLPGTAPKLLIY

5 **CDR L2**

GNDQRPS GVPDRFSGSKSGTSASLAITGLQAEDEADYYC

CDR L3

QSYDRGTHPALL FGTGTKVTVLG (SEQ ID NO:24)

10

CDR residues are assigned according to the Kabat definitions.

EXAMPLE 2: Mutation of Y61 at Hypermutation and Contact Positions

15 Typically selection of recombinant antibodies with improved affinities can be carried out using phage display methods. This is accomplished by randomly mutating combinations of CDR residues to generate large libraries containing single-chain antibodies of different sequences. Typically, antibodies with improved affinities are selected based on their ability to reach an equilibrium in an antibody-antigen reaction.

20 However, when Y61 scFV was expressed on phage surface and incubated with IL-12, selection conditions could not be found that would allow the system to reach normal antibody-antigen equilibrium. The scFV-phage remained bound to IL-12, presumably due to a non-specific interaction, since purified Y61 scFv exhibits normal dissociation kinetics. Since the usual methods of phage-display affinity maturation to Y61 (i.e.

25 library generation and selections by mutagenesis of multiple CDR residues) could not be utilized, a new strategy was developed in which individual CDR positions were mutated.

This strategy involves selection of appropriate CDR positions for mutation and is based on identification and selection of amino acids that are preferred selective mutagenesis positions, contact positions, and/or hypermutation positions. Contact

30 positions are defined as residues that have a high probability of contact with an antigen when the antigen interacts with the antibody, while hypermutation positions are defined as residues considered to have a high probability for somatic hypermutation during *in vivo* affinity maturation of the antibody. Preferred selective mutagenesis positions are CDR positions that are both contact and hypermutation positions. The Y61 antibody

35 was already optimized in the CDR3 regions using the procedure described in Example 1, therefore it was difficult to further improve the area which lies at the center of the antibody binding site using phage-display selection methods. Greater improvements in activity were obtained by mutation of potential contact positions outside the CDR3 regions by either removing a detrimental antigen-antibody contact or, engineering a new

40 contact.

Amino acids residues of Y61 which were considered contact points with antigen, and those CDR positions which are sites of somatic hypermutations during *in vivo* affinity maturation, are shown in Table 3 (see Appendix A). For Y61 affinity maturation, 15 residues outside CDR3, 3 residues within the L3 loop, and 5 residues in the H3 loop were selected for PCR mutagenesis.

Y61 scFv gene was cloned into the pUC119(Sfi) plasmid vector for mutagenesis. Oligonucleotides were designed and synthesized with randomized codons to mutate each selected position. Following PCR mutagenesis, a small number of clones (~24) were sequenced and expressed in a host cell, for example, in a bacterial, yeast or mammalian host cell. The expressed antibody was purified and the k_{off} measured using the BIAcore system. Clones with improved off-rates, as compared to Y61, were then tested in neutralization assays. This procedure was repeated for other CDR positions. Individual mutations shown to have improved neutralization activity were combined to generate an antibody with even greater neutralization potency.

The Y61 CDR positions that were mutated in order to improve neutralization potency, and the respective amino-acid substitutions at each position are shown in Figures 2A-2H. Off-rates, as determined by BIAcore analysis, are given. These off rates are also shown in the histograms to the right of each table.

Results of these substitutions at positions H30, H32, H33, H50, H53, H54, H58, H95, H97, H101, L50, L92, L93, demonstrated that all amino-acid substitutions examined resulted in antibodies with poorer off-rates than Y61. At positions H52, L32, and L50, only a one amino acid substitution was found to improve the off-rate of Y61, all other changes adversely affected activity. For L50, this single Gly→Tyr change significantly (5-10 times) improved the neutralization potency of Y61. The results demonstrated the importance of these positions to Y61 activity, and suggest that in most cases phage-display was able to select for the optimal residues. However, at positions H31, H56, L30, and L94, several substitutions were found to improve Y61 off-rate, suggesting that these positions were also important for antigen binding, although the phage display approach did not allow selection of the optimal residues.

Selective mutation of contact and hypermutation positions of Y61 identified amino acid residue L50 in the light chain CDR2, and residue L94 of the light chain CDR3, which improved the neutralization ability of Y61. A combination of these mutations produced an additive effect, generating an antibody, J695, that exhibited a significant increase in neutralization ability. The full length sequence of J695 heavy and light chain variable region sequences is shown below.

CDR H1

5

FIRYDGSNKYYADSVKG RFTISRDN SKNTLYLQMNSLRAEDTAVYYCKT

10 **HGSHDN** WGQGTMTVTVSS (SEQ ID NO: 31)

CDR L1

15

YNDQRPS GVPDRFSGSKSGTSASLAITGLQAEDEADYYC

OSYDRYTHPALL FGTGTVLVG (SEQ ID NO: 32)

25 CDR residues are assigned according to the Kabat definitions.

A summary of the heavy and light chain variable region sequence alignments showing the lineage development of clones that were on the path from Joe9 to J695 is shown in Figures 1A-1D. The CDRs and residue numbering are according to Kabat.

EXAMPLE 3: Functional Activity of Anti-hIL-12 Antibodies

To examine the functional activity of the human anti-human IL-12 antibodies of the invention, the antibodies were used in several assays that measure the ability of an antibody to inhibit IL-12 activity.

A. Preparation of Human PHA-activated Lymphoblasts

Human peripheral blood mononuclear cells (PBMC) were isolated from a leukopac collected from a healthy donor by Ficoll-Hypaque gradient centrifugation for 45 minutes at 1500 rpm as described in Current Protocols in Immunology, Unit 7.1. PBMC at the interface of the aqueous blood solution and the lymphocyte separation medium were collected and washed three times with phosphate-buffered saline (PBS) by centrifugation for 15 minutes at 1500 rpm to remove Ficoll-Paque particles.

The PBMC were then activated to form lymphoblasts as described in Current Protocols in Immunology, Unit 6.16. The washed PBMC were resuspended at $0.5-1 \times 10^6$

cells/ml in RPMI complete medium (RPMI 1640 medium, 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin), supplemented with 0.2% (v/v) PHA-P (Difco, Detroit, MI) and cultured for four days at 37°C in a 5% CO₂ atmosphere. After four days, cell cultures were split 1:1 by volume in RPMI complete medium, plus
 5 0.2% (v/v) PHA-P and 50 U/ml recombinant human IL-2. Recombinant human IL-2 was produced by transfection of an expression vector carrying the human IL-2 cDNA into COS cells (see Kaufman *et al.*, (1991) *Nucleic Acids Res.* 19, 4484-4490), and purified as described in PCT/US96/01382. Cell cultures were then incubated for an additional one to three days. PHA blast cells were harvested, washed twice with RPMI
 10 complete medium and frozen in 95% FBS, 5% DMSO at 10x10⁶ cells/ml.

PHA blast cells to be used for the IL-12 receptor binding assay (see section B) were collected after one day culture in the presence of IL-2, whereas PHA blast cells to be used for the PHA blast proliferation assay (see section C) and the interferon-gamma induction assay (see section D) were collected after three day culture in the presence of
 15 IL-2.

B. IL-12 Receptor Binding Assay

The ability of anti-IL-12 antibodies to inhibit binding of radiolabelled IL-12 to IL-12 receptors on PHA blasts were analyzed as follows. Various concentrations of
 20 anti-IL-12 antibody were preincubated for 1 hour at 37°C with 50-100 pM ¹²⁵I-hIL-12 (iodinated hIL-12 was prepared using the Bolton-Hunter labeling method to a specific activity of 20-40mCi/mg from NEN-Dupont) in binding buffer (RPMI 1640, 5% FBS, 25 mM Hepes pH 7.4). PHA blast cells isolated as described above, were washed once and resuspended in binding buffer to a cell density of 2x10⁷ cells/ml. PHA blasts (1x10⁶
 25 cells) were added to the antibody ¹²⁵I-hIL-12 mixture and incubated for two hours at room temperature. Cell bound radioactivity was separated from free ¹²⁵I-hIL-12 by centrifugation of the assay mixture for 30 seconds at room temperature, aspiration of the liquid and a wash with 0.1ml binding buffer, followed by centrifugation at 4°C for 4 min at 10,000 x g. The cell pellet was examined for cell bound radioactivity using a gamma
 30 counter. Total binding was determined in the absence of antibody and non-specific binding was determined by inclusion of 25 nM unlabeled IL-12 in the assay. Incubations were carried out in duplicate.

In the IL-12 receptor binding assay using the Y61 and J695 human anti-IL-12 antibodies, both antibodies demonstrated a comparable inhibition of IL-12 receptor
 35 binding. Y61 inhibited IL-12 receptor binding with an IC₅₀ value of approximately 1.6 x 10⁻¹¹M, while J695 had an IC₅₀ value of approximately 1.1 x 10⁻¹¹M.

C. Human PHA Blast Proliferation Assay

Anti-IL-12 antibodies were evaluated for their ability to inhibit PHA blast proliferation (which proliferation is stimulated by IL-12). Serial dilutions of anti-IL-12 antibody were preincubated for 1 hour at 37°C, 5% CO₂ with 230 pg/ml hIL-12 in
 5 100 ml RPMI complete medium in a microtiter plate (U-bottom, 96-well, Costar, Cambridge, MA). PHA blast cells isolated as described above, were washed once and resuspended in RPMI complete medium to a cell density of 3×10^5 cells/ml. PHA blasts (100 ml, 3×10^4 cells) were added to the antibody/hIL-12 mixture, incubated for 3 days at 37°C, 5% CO₂ and labeled for 4-6 hours with 0.5 mCi/well (3H)-Thymidine
 10 (Amersham, Arlington Heights, IL). The culture contents were harvested onto glass fiber filters by means of a cell harvester (Tomtec, Orange, CT) and (³H)-Thymidine incorporation into cellular DNA was measured by liquid scintillation counting. All samples were assayed in duplicate.

The results of neutralization in the presence of varying concentrations of p70:p40
 15 (i.e. the ratio of IL-12 heterodimer to free p40 subunit) is shown in Table 4 (see Appendix A).

Analysis of the Y61 human anti-IL-12 antibody in the PHA blast proliferation assay demonstrated that the antibody inhibited PHA blast proliferation with an IC₅₀ value of approximately 1.8×10^{-10} M in the presence of IL-12 p70 alone, without any
 20 excess p40 (p70:p40 ratio of 1:0). In the presence of a 50-fold excess of free p40 (p70:p40 at a ratio of 1:50), the Y61 antibody inhibited PHA blast proliferation with an IC₅₀ value of approximately 1.8×10^{-10} M. This result demonstrates that the ability of Y61 to inhibit blast proliferation is not compromised by the presence of excess p40.

The human anti-IL-12 antibody, J695 inhibited PHA blast proliferation with an
 25 IC₅₀ value of approximately 1.0×10^{-11} M in the presence of p70:p40 at a ratio of 1:0. In the presence of a p70:p40 ratio of 1:50, this antibody inhibited PHA blast proliferation with an IC₅₀ value of approximately $5.8 \pm 2.8 \times 10^{-12}$ M (n=2), demonstrating that the excess p40 had only a slight inhibitory effect on the antibody. Overall results demonstrate the improved neutralization activity of J695 in comparison with Y61 due to
 30 the mutations at L50 and L94.

D. Interferon-gamma Induction Assay

The ability of anti-IL-12 antibodies to inhibit the production of IFN γ by PHA blasts (which production is stimulated by IL-12) was analyzed as follows. Various
 35 concentrations of anti-IL-12 antibody were preincubated for 1 hour at 37°C, 5% CO₂ with 200-400 pg/ml hIL-12 in 100 ml RPMI complete medium in a microtiter plate (U-bottom, 96-well, Costar). PHA blast cells isolated as described above, were washed once and resuspended in RPMI complete medium to a cell density of 1×10^7 cells/ml.

PHA blasts (100 μ l of 1×10^6 cells) were added to the antibody/hIL-12 mixture and incubated for 18 hours at 37°C and 5% CO₂. After incubation, 150 μ l of cell free supernatant was withdrawn from each well and the level of human IFN γ produced was measured by ELISA (Endogen Interferon gamma ELISA, Endogen, Cambridge, MA).

- 5 Each supernatant was assayed in duplicate.

Analysis of human anti-hIL-12 antibody, Y61 in this assay demonstrated that Y61 inhibited human IFN γ production with an IC₅₀ value of approximately 1.6×10^{-10} M, while the human anti-IL-12 antibody, J695, inhibited human IFN γ production with an IC₅₀ value of approximately $5.0 \pm 2.3 \times 10^{-12}$ M (n=3). The result demonstrates the
 10 substantial improvement in the affinity of J695 as a result of the modifications at L50 and L94.

E. Induction of Non-human IL-12 from Isolated PBMC

To examine the cross-reactivity of the human anti-hIL-12 antibodies with IL-12
 15 from other species, non-human IL-12 was produced as follows. PBMC were separated from fresh heparinized blood by density gradient centrifugation as described above using lymphoprep (Nycomed, Oslo, Norway) for cynomolgus monkey, baboon, and dog, PBMC, Accu-paque (Accurate Chemical & Sci. Corp., Westbury, NY) for dog PBMC or Lympholyte-rat (Accurate Chemical & Sci. Corp., Westbury, NY) for rat PBMC.

20 The PBMC were then induced to produce IL-12 as described (D'Andrea *et al.*, (1992) *J. Exp. Med.* 176, 1387-1398, Villinger *et al.*, (1995) *J. Immunol.* 155, 3946-3954, Buettner *et al.*, (1998) *Cytokine* 10, 241-248). The washed PBMC were resuspended at 1×10^6 cells/ml in RPMI complete medium, supplemented with 0.0075% (wt/vol) of SAC (Pansorbin; Calbiochem-Behring Co., La Jolla, CA) or 1-5 mg/ml
 25 ConA (Sigma Chemical Co., St. Louis, MO) plus 0.0075% SAC and incubated for 18 hours at 37°C in a 5% CO₂ atmosphere. Cell-free and SAC-free medium was collected by centrifugation and filtering through 0.2 mm filters.

IL-12 from the rhesus monkey was obtained as recombinant rhesus IL-12 from Emory University School of Medicine, Atlanta, GA.

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F. Murine 2D6 Cell Proliferation Assay

The murine T cell clone 2D6 proliferates in response to murine IL-2, IL-4, IL-7 and IL-12 (Maruo *et al.*, (1997) *J. Leukocyte Biol.* 61, 346-352). A significant proliferation was also detected in response to rat PBMC supernatants containing rat IL-
 35 12. The cells do not respond to dog, cynomolgus, baboon or human IL-12. Murine 2D6 cells were propagated in RPMI complete medium supplemented with 50 mM beta-mercaptoethanol (β ME) and 30 ng/ml murine IL-12. One day prior to the assay, the

murine IL-12 was washed out and the cells were incubated overnight in RPMI complete medium plus β ME.

Serial dilutions of anti-IL-12 antibody were preincubated for 1 hour at 37°C, 5% CO₂ with 40 pg/ml murine IL-12 in 100 ml RPMI complete medium plus β ME in a microtiter plate (U-bottom, 96-well, Costar). 2D6 cells were washed once and resuspended in RPMI complete medium containing β ME to a cell density of 1×10^5 cells/ml. 2D6 cells (100 μ l, 1×10^4 cells) were added to the antibody/hIL-12 mixture, incubated for 3 days at 37°C, 5% CO₂ and labeled for 4-6 hours with 0.5 mCi/well (3H)-Thymidine. The culture contents were harvested and counted by liquid scintillation counting. All samples were assayed in duplicate.

G. Species Cross-reactivity of J695 with Non-Human IL-12

Species cross-reactivity of J695 with non-human IL-12 was analyzed using PBMC's isolated from several non-human species. The presence of non-human IL-12 activity in the rat, dog, cynomolgus and baboon PBMC supernatants was confirmed using several bioassays described above, such as the murine 2D6 cell proliferation assay, the human PHA blast proliferation assay and the interferon-gamma induction assay by blocking the non-human PBMC induced responses with rabbit and/or sheep polyclonal antibodies to murine and/or human IL-12. Cross-reactivity of the human anti-hIL-12 antibodies Y61 and J695 with non-human IL-12 in PBMC supernatants or purified murine and rhesus IL-12 was then assessed in the same bioassay(s) by determining the J695 antibody concentration at which 50% inhibition of the response was observed. The species cross-reactivity results are summarized in Table 5. The results demonstrate that Y61 and J695 are each able to recognize IL-12 from monkeys (e.g, cynomolgus and rhesus IL-12 for Y61, and cynomolgus, rhesus and baboon for J695) and that J695 is approximately 35 fold less active on dog IL-12; neither Y61 nor J695 cross reacts with mouse or rat IL-12.

H. Human cytokine specificity of J695

The specificity of J695 was tested in a competition ELISA in which a panel of human cytokines was tested for their ability to interfere with the binding of soluble J695 to immobilized human IL-12. The panel of human cytokines included IL-1 α and IL-1 β (Genzyme, Boston, MA), IL-2 (Endogen), IL-4, IL-10, IL-17, IFN-gamma, and TGF- β 1 (R&D, Minneapolis, MN) IL-8 (Calbiochem), PDGF, IGF-I, and IGF-II (Boehringer Mannheim Corp., Indianapolis, IN), TNF α and lymphotoxin, IL-6, soluble IL-6 receptor, IL-11, IL-12 p70, IL-12 p40, M-CSF, and LIF. EBI-3, an IL-12 p40 related protein that is induced by Epstein-Barr virus infection in B lymphocytes (Devergne *et*

Table 5 Species Cross Reactivity Data

IC ₅₀ (M)								
Antibody		Mouse IL-12	Rat IL-12	Dog IL-12	Cyno IL-12	Rhesus IL-12	Baboon IL-12	Human IL-12
Name	Specificity	Purified	PBMC sup	PBMC sup	PBMC sup	Purified	PBMC sup	Purified
C17.15	rat- α mulIL12	3.0 x 10 ⁻¹¹						
R03B03	rabbit- α mulIL12	1.5 x 10 ⁻¹⁰	6.0 x 10 ⁻¹⁰					
C8.6.2	mouse- α mulIL12				1.2 x 10 ⁻¹⁰	1.0 x 10 ⁻¹⁰	2.0 x 10 ⁻¹⁰	5.0 x 10 ⁻¹¹
Y61	human- α mulIL12	Non-neutralizing			2.2 x 10 ⁻¹⁰	1.0 x 10 ⁻¹⁰		1.7 x 10 ⁻¹⁰
J695	human- α mulIL12	Non-neutralizing	Non-neutralizing	3.5 x 10 ⁻¹⁰	1.0 x 10 ⁻¹¹	1.0 x 10 ⁻¹¹	1.5 x 10 ⁻¹¹	5.0 x 10 ⁻¹²

al., (1996) *J. Virol.* 70, 1143-1153) was expressed as a human IgG-Fc chimera (EBI-3/Fc) Single-stranded salmon sperm DNA (Sigma) was also tested.

Flat-bottom ELISA immunoassay microtiter plates (96 well, high binding, Costar) were coated overnight at 4°C with 0.1 ml human IL-12 (2 µg/ml in 0.1 M carbonate coating buffer (4 volumes 0.1 M NaHCO₃ plus 8.5 volumes 0.1 M NaHCO₃)). The plates were washed twice with PBS containing 0.05 % Tween 20 (PBS-T), blocked with 200µl of 1 mg/ml bovine serum albumin (BSA, Sigma) in PBS-T for 1 hour at room temperature, and again washed twice with PBS-T. Samples (100 µl) containing IL-12 antibody J695 (100 ng/ml) and each cytokine (2nM) in PBS-T containing 50 µg/ml BSA (PBS-T/BSA) were added and incubated for 2 h at room temperature. The plates were washed 4 times and incubated for 1h at room temperature with 100 µl mouse anti-human lambda-HRP (1:500 in PBS-T/BSA, Southern Biotech. Ass. Inc., Birmingham, AL). The plates were washed 4 times and developed with ABTS (Kirkegaard & Perry Lab., Gaithersburg, MD) for 20-30 minutes in the dark. The OD_{450nm} was read using a microplate reader (Molecular Devices, Menlo Park, CA). Percent binding was determined relative to J695 binding to the IL-12 coated plate in the absence of any soluble cytokine.

The results demonstrated that J695 binding to immobilized human IL-12 was blocked only by human IL-12 p70 and to a lesser extent, by human IL-12 p40 and not by any of the other cytokines tested.

I. Binding to a Novel IL-12 Molecule

An alternative IL-12 heterodimer has been described, in which the p35 subunit is replaced by a novel p19 molecule. P19 was identified using 3D homology searching for IL-6/IL-12 family members, and is synthesized by activated dendritic cells. P19 binds to p40 to form a p19/p40 dimer, which has IL-12 –like activity, but is not as potent as the p35/p40 heterodimer in IFNγ induction. Antibodies which recognize p40 alone, but preferably in the context of a p70 molecule (e.g., J695 and Y61, see Example 3H) are expected to also neutralize both the p35/p40 molecules and the p19/p40 molecules.

EXAMPLE 4: In vivo Activity of Anti-hIL-12 Antibodies

The *in vivo* effects of IL-12 antibodies on IL-12 induced responses were examined in a model modified from one used by Bree *et al.* to study the effect of human IL-12 on peripheral hematology in cynomolgus monkey Bree *et al.*, (1994) *Biochem Biophys Res. Comm.* 204: 1150-1157. In those previous studies, administration of human IL-12 at 1 µg/kg/day for a period of 5 days resulted in a decrease in white blood cell count (WBC), especially in the lymphocyte and monocyte subsets after 24 hours. A

decrease in the platelet count was observed at 72 hours. Levels of plasma neopterin, a marker of monocyte activation in response to IFN- γ , began to elevate at 24 hours and were the highest at 72 hours.

In the first study with human anti-hIL-12 antibodies, fifteen healthy cynomolgus monkeys with an average weight of 5kg, were sedated and divided into 5 groups (n=3). Group 1 received an intravenous (IV) administration of 10 mg/kg human intravenous immunoglobulin (IVIG, Miles, Eckhart, IN, purified using protein A Sepharose). Group 2 received an intravenous administration of 1 mg/kg C8.6.2 (neutralizing mouse anti-human IL-12 monoclonal antibody). Group 3 received an intravenous administration of 10 mg/kg C8.6.2. Group 4 received an intravenous administration of 1 mg/kg Y61 (human anti-human IL-12 antibody, purified from CHO cell conditioned medium). Group 5 received an intravenous administration of 10 mg/kg Y61.

One hour after the antibody administration all animals received a single subcutaneous (SC) injection of human IL-12 (1 μ g/kg). Blood samples were taken at the following time points: baseline, 8, 24, 48, 96 and 216 hours, and analyzed for complete blood cell counts with differentials and serum chemistry. Serum human IL-12, C8.6.2 antibody, Y61 antibody, monkey IFN-gamma, monkey IL-10, monkey IL-6 and plasma neopterin levels were also measured.

Animals treated with IL-12 plus IVIG control antibody (Group 1) showed many of the expected hematological changes, including decreases in WBC, platelets, lymphocyte count and monocyte count. These decreases were not seen or were less pronounced in the animals treated with either the C8.6.2 or Y61 antibody at 1 or 10 mg/kg (Groups 2-5).

Serum or plasma samples were analyzed by ELISA specific for monkey IFN-gamma and monkey IL-10 (Biosource International, Camarillo, CA), monkey IL-6 (Endogen) and plasma neopterin (ICN Pharmaceuticals, Orangeburg, NY). IFN-gamma, IL-10 or IL-6 were not detected in any of the IL-12 treated animals including the control animals treated with IL-12 plus IVIG. This was probably due to the low level exposure to IL-12 (only 1 dose of 1 μ g/kg). Nevertheless, plasma neopterin levels increased about three fold in the IL-12 plus IVIG treated animals but did not change in all C8.6.2 or Y61 treated animals, including the lower dose (1 mg/kg) Y61 treated animals, indicating that Y61 was effective *in vivo* in blocking this sensitive response to IL-12.

In a second study, *in vivo* activity and pharmacodynamics (PD) of J695 in cynomolgous monkeys were studied by administering exogenous rhIL-12 and determining if J695 could block or reduce the responses normally associated with rhIL-12 administration. Male cynomolgus monkeys (n=3 per group) were administered a single dose of 0.05, 0.2, or 1.0 mg/kg J695 or 1 mg/kg intravenous immunoglobulin

(IVIG) as a bolus intravenous (IV) injection via a saphenous vein or subcutaneously (SC) in the dorsal skin. One hour following the administration of J695 or IVIG, all animals received a single SC dose of 1 µg/kg rhIL-12 in the dorsal skin. Blood samples were collected via the femoral vein up to 28 days after J695 administration. Serum was acquired from each blood sample and assayed for IL-12, J695, IFN-γ, and anti-J695 antibodies by ELISA. Neopterin was assayed by reverse-phase high performance liquid chromatography.

The levels of neopterin, normalized with respect to the levels of neopterin that were measured before administration of J695 or rhIL-12, are shown in Figure 3. To compare the suppression of neopterin between groups, the area under the curve (AUC) normalized for neopterin levels was calculated for each animal (Table 6). Neopterin exposure (AUC) was suppressed in a dose-dependent manner between approximately 71 and 93% in the IV groups and between 71 and 100% in SC groups, relative to the IVIG control groups. These results suggest that the dose of J695 necessary for 50% inhibition of the neopterin response (ED₅₀) was less than 0.05 mg/kg when administered by either the IV or SC route.

Table 6: Dose-Dependent Suppression of IL-12 Induced Neopterin by J695 in Cynomolgus Monkeys

Route of dosing IVIG or J695 and rhIL-12	J695 Dose (mg/kg)	IVIG Dose (mg/kg)	AUC of Normalized Neopterin Levels	% Reduction of Neopterin AUC Compared with Control
Single IV injection followed 1 hr later by a dose of 1µg/kg human IL-12 given SC	-	1.0	1745 ± 845	0
	0.05	-	502 ± 135	71.3
	0.2	-	199 ± 316	88.6
	1.0	-	128 ± 292	92.7
Single SC injection followed 1 hour later by a dose of 1 µg/kg human IL-12 given SC	-	1.0	1480 ± 604	0
	0.05	-	426 ± 108	71.2
	0.2	-	395 ± 45.9	73.3
	1.0	-	0 ± 109	100

Treatment with J695 also prevented or reduced the changes in hematology normally associated with rhIL-12 administration (leukopenia and thrombocytopenia). At 24 hours after rhIL-12 administration lymphocyte counts were reduced by approximately 50% when compared to baseline values in the control IV and SC IVIG treated groups. Administration of J695 either SC or IV at all three dose levels prevented this reduction, resulting in lymphocyte counts at 24 hours approximately the same as

baseline values. At 48 hours after IL-12 administration, platelet counts in the groups treated with IV and SC IVIG were reduced by approximately 25% when compared to baseline values.

An example dose schedule targeted to maintain serum levels above the 90% effect level would be 1 mg/kg IV and SC given approximately every other week, or 0.3 mg/kg given approximately every week, assuming slight accumulation during repeated dosing. This study demonstrates that antibody can be given safely to monkeys at such dosages. In independent toxicity studies, it was further found that up to 100 mg/kg of the antibody can be given safely to monkeys.

J695 was also effective in preventing IFN- γ production in mice treated with a chimeric IL-12, a molecule which combines the murine p35 subunit with the human IL-12 p40 subunit. In contrast to human IL-12 which is biologically inactive in mice, this chimeric IL-12 retains biological function in mice, including induction of IFN- γ . In addition, the human p40 subunit allows the molecule to be bound and neutralized by J695. Chimeric IL-12 at a dose of 0.05 mg/kg i.p. was administered to female C3H/HeJ mice (10/experimental group) in five daily doses on days 0, 1, 2, 3, and 4. J695 was given on days 0, 2 and 4 at doses of 0.05, 0.01, 0.002, 0.0004, 0.00008, and 0.000016 mg/kg i.p., 30' prior to the IL-12 injections. The control hulgG1 γ was given IP. at a dose of 0.05 mg/kg on days 0, 2, and 4. The mice were bled on day 5, and serum IFN- γ levels were determined by ELISA. The results demonstrated that J695 caused dose-dependent inhibition of IFN- γ production with an ED₅₀ of approximately 0.001 mg/kg. Collectively, these results demonstrate that J695 is a potent inhibitor of IL-12 activity *in vivo*.

EXAMPLE 5: Kinetic Analysis of Binding of Human Antibodies to Recombinant human IL-12 (rhIL-12)

Real-time binding interactions between captured ligand (human anti-rhIL-12 antibody J695, captured on a biosensor matrix) and analyte (rhIL12 in solution) were measured by surface plasmon resonance (SPR) using the BIAcore system (Biacore AB, Uppsala, Sweden). The system utilizes the optical properties of SPR to detect alterations in protein concentration within a dextran biosensor matrix. Proteins are covalently bound to the dextran matrix at known concentrations. Antibodies are injected through the dextran matrix and specific binding between injected antibodies and immobilized ligand results in an increased matrix protein concentration and resultant change in the SPR signal. These changes in SPR signal are recorded as resonance units (RU) and are displayed with respect to time along the y-axis of a sensorgram.

To facilitate immobilization of goat anti-human IgG (Southern Biotechnology Associates, Cat. No. 2040-01, Birmingham, AL) on the biosensor matrix, goat anti-human IgG is covalently linked via free amine groups to the dextran matrix by first activating carboxyl groups on the matrix with 100 mM N-hydroxysuccinimide (NHS) and 400 mM N-Ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). Next, goat anti-human IgG is injected across the activated matrix. Thirty-five microliters of goat anti-human IgG (25 µg/ml), diluted in sodium acetate, pH 4.5, is injected across the activated biosensor and free amines on the protein are bound directly to the activated carboxyl groups. Unreacted matrix EDC-esters are deactivated by an injection of 1 M ethanolamine. Standard amine coupling kits were commercially available (Biacore AB, Cat. No. BR-1000-50, Uppsala, Sweden).

J695 was diluted in HBS running buffer (Biacore AB, Cat. No. BR-1001-88, Uppsala, Sweden) to be captured on the matrix via goat anti-human IgG. To determine the capacity of rhIL12-specific antibodies to bind immobilized goat anti-human IgG, a binding assay was conducted as follows. Aliquots of J695 (25 µg/ml; 25 µl aliquots) were injected through the goat anti-human IgG polyclonal antibody coupled dextran matrix at a flow rate of 5 µl/min. Before injection of the protein and immediately afterward, HBS buffer alone flowed through each flow cell. The net difference in signal between the baseline and the point corresponding to approximately 30 seconds after completion of J695 injection was taken to represent the amount of IgG1 J695 bound (approximately 1200 RU's). Direct rhIL12 specific antibody binding to soluble rhIL12 was measured. Cytokines were diluted in HBS running buffer and 50 µl aliquots were injected through the immobilized protein matrices at a flow rate of 5 µl/min. The concentrations of rhIL-12 employed were 10, 20, 25, 40, 50, 80, 100, 150 and 200 nM. Prior to injection of rhIL-12, and immediately afterwards, HBS buffer alone flowed through each flow cell. The net difference in baseline signal and signal after completion of cytokine injection was taken to represent the binding value of the particular sample. Biosensor matrices were regenerated using 100 mM HCl before injection of the next sample. To determine the dissociation constant (off-rate), association constant (on-rate), BIAcore kinetic evaluation software (version 2.1) was used.

Representative results of CHO derived J695 binding to rhIL-12 as compared to the COS derived J695, are shown in Table 7.

Table 7: Binding of CHO or COS derived J695 to rhIL-12.

Source	rhIL12, nM	rhIL12 bound, RU's	Ab, bound, RU's	rhIL12/AB
CHO	200	1112	1613	1.48
CHO	150	1033	1525	1.45

CHO	100	994	1490	1.43
CHO	80	955	1457	1.40
CHO	50	912	1434	1.36
CHO	40	877	1413	1.33
CHO	25	818	1398	1.25
CHO	20	773	1382	1.20
CHO	10	627	1371	0.98
Source	rhIL12, nM	rhIL12 bound, RU's	Ab, bound, RU's	rhIL12/AB
COS	200	1172	1690	1.49
COS	150	1084	1586	1.46
COS	100	1024	1524	1.44
COS	80	985	1489	1.42
COS	50	932	1457	1.37
COS	40	894	1431	1.34
COS	25	833	1409	1.27
COS	20	783	1394	1.20
COS	10	642	1377	1.00

Molecular kinetic interactions between captured J695 and soluble rhIL-12 were quantitatively analyzed using BIAcore technology. Several independent experiments were performed and the results were analyzed by the available BIAcore mathematical analysis software to derive kinetic rate constants, as shown in Table 8.

Table 8: Apparent kinetic rate and affinity constants of J695 for rhIL-12.

Antibody	Source	On-rate (M ⁻¹ s ⁻¹), Avg.	Off-rate (s ⁻¹), Avg.	Kd (M), Avg.
J695	CHO	3.52E+05	4.72E-05	1.34E-10
J695	COS	3.40E+05	2.61E-05	9.74E-11

There was a small difference between the calculated apparent constant (Kd) for the interaction between CHO derived J695 ($K_d = 1.34 \times 10^{-10} \text{M}^{-1}$) and COS derived J695 ($K_d = 9.74 \times 10^{-11} \text{M}^{-1}$) antibodies. The apparent dissociation constant (Kd) between J695 and rhIL12 was estimated from the observed rate constants by the formula: $K_d = \text{off-rate} / \text{on-rate}$.

To determine the apparent association and dissociation rate constant for the interaction between J695 and rhIL-12, several binding reactions were performed using a

fixed amount of J695 (2 $\mu\text{g/ml}$) and varying concentrations of rhIL-12. Real-time binding interaction sensorgrams between captured J695 and soluble rhIL12 showed that both forms of antibody were very similar for both the association and dissociation phase.

To further evaluate the capacity of captured IgG1 J695 mAb to bind soluble recombinant cytokine, a direct BIAcore method was used. In this method, goat anti-human IgG (25 $\mu\text{g/ml}$) coupled carboxymethyl dextran sensor surface was coated with IgG1 J695 (2 $\mu\text{g/ml}$) and recombinant cytokine was then added. When soluble rhIL12 was injected across a biosensor surface captured with CHO or COS derived IgG1 J695, the amount of signal increased as the concentration of cytokine in the solution increased. No binding was observed with rmIL12 (R&D Systems, Cat. No. 419-ML, Minneapolis, MN) or rh IL12 any concentration tested up to 1000 nM. These results support the conclusion that IgG1 J695 antibodies recognize a distinct determinant on rhIL-12.

Table 9 shows the results of an experiment using BIAcore to demonstrate human IgG1 J695 mAb binding to only soluble rhIL12 and none of the other recombinant cytokines.

Table 9: Epitope mapping of J695 using BIAcore technology.

	Captured ligand COS J695	Captured ligand CHO J695
Soluble analyte		
rec. human IL12	Positive	Positive
rec. murine IL12	Negative	Negative

20 **EXAMPLE 6: Further Studies of J695 Affinity for IL-12**

Molecular kinetic interactions between J695 antibody and human IL-12 were quantitatively analyzed using BIAcore plasmon resonance technology, and apparent kinetic rate constants were derived.

BIAcore technology was used to measure the binding of soluble rhIL-12 to solid phase captured J695. A goat anti-human IgG antibody was immobilized on the biosensor chips, then a fixed amount of J695 was injected and captured on the surface. Varying concentrations of rhIL-12 were applied, and the binding of IL-12 at different concentrations to J695 was measured as a function of time. Apparent dissociation and association rate constants were calculated, assuming zero-order dissociation and first order association kinetics, as well as a simple one-to-one molecular interaction between J695 and IL-12. Three independent experiments were performed, and the values shown are averages for the three experiments. From these measurements, the apparent dissociation (k_d) and association (k_a) rate constants were derived and used to calculate a K_d value for the interaction (see Table 10). The results indicated that J695 has a high affinity for rhIL-12.

Table 10: Kinetic Parameters for the Interaction Between J695 and Human IL-12

Kinetic Parameter	Value
k_d	$3.71 \pm 0.40 \times 10^{-5} \text{ s}^{-1}$
k_a	$3.81 \pm 0.48 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$
K_d	$9.74 \times 10^{-11} \text{ M (14 ng/mL)}$

5

EXAMPLE 7: Characteristics and Neutralization Activity of C17.15, a Rat Monoclonal Antibody to Murine Interleukin-12

To assess the relevance of IL-12 treatment studies in mouse models of inflammation and autoimmunity using monoclonal antibodies specific for murine IL-12 to similar approaches in human disease, the interaction of C17.15, a rat anti-murine IL-12 monoclonal antibody with murine IL-12, was examined. The ability of C17.15 to neutralize murine IL-12 activity in a PHA blast proliferation assay, and to block murine IL-12 binding to cell surface receptors, was assessed, as were the kinetics of the C17.15-murine IL-12 binding interaction.

In a human PHA blast proliferation assay (See Example 3), serial dilutions of C17.15 or rat IgG2a (a control antibody) were preincubated with 230 pg/mL murine IL-12 for 1 hr at 37 °C. PHA-stimulated blast cells were added to the antibody-IL-12 mixtures and incubated for 3 days at 37 °C. The cells were subsequently labeled for 6 h with 1 $\mu\text{Ci/well}$ [^3H]-thymidine. The cultures were harvested and [^3H]-thymidine incorporation was measured. Background non-specific proliferation was measured in the absence of added murine IL-12. All samples were assayed in duplicate. The IC_{50} (M) of C17.15 for recombinant murine IL-12 in this assay was found to be 1.4×10^{-11} , as compared to the IC_{50} value of 5.8×10^{-12} observed for J695 for recombinant human IL-12 under the same conditions (see Table 11).

Table 11: Comparison of the properties of anti-human IL-12 monoclonal antibody J695 and the rat anti-mouse IL-12 monoclonal antibody C17.15

Antibody	Epitope	Biomolecular Interaction Assay			Receptor Binding Assay	PHA blast Assay
		k_a , on-rate ($\text{M}^{-1} \text{s}^{-1}$)	k_d , off-rate (s^{-1})	K_d (M)		
J695	Hu p40	3.81×10^5	3.71×10^{-5}	9.74×10^{-11}	1.1×10^{-11}	5.8×10^{-12}
C17.15	Mu p40	3.80×10^5	1.84×10^{-4}	4.80×10^{-10}	1.5×10^{-10}	1.4×10^{-11}

30

The ability of C17.15 to inhibit the binding of murine IL-12 to cellular receptors was also measured. Serial dilutions of C17.15 were pre-incubated for 1 hr at 37 °C with 100 pM [¹²⁵I]-murine IL-12 in binding buffer. The 2D6 cells (2x10⁶) were added to the antibody/[¹²⁵I]-murine IL-12 mixture and incubated for 2 hours at room temperature.

- 5 Cell-bound radioactivity was separated from free [¹²⁵I]-IL-12, and the remaining cell-bound radioactivity was determined. Total binding of the labeled murine IL-12 to receptors on 2D6 cells was determined in the absence of antibody, and non-specific binding was determined by the inclusion of 25 nM unlabelled murine IL-12 in the assay. Specific binding was calculated as the total binding minus the non-specific binding.
- 10 Incubations were carried out in duplicate. The results showed that C17.15 has an IC₅₀ (M) of 1.5x10⁻¹⁰ for inhibition of binding of murine IL-12 to cellular receptors.

- The affinity of C17.15 for recombinant murine IL-12 was assessed by biomolecular interaction analysis. A goat anti-rat IgG antibody was immobilized on the biosensor chips, followed by an injection of a fixed amount of the C17.15 antibody, resulting in capture of C17.15 on the surface of the chip. Varying concentrations of recombinant murine IL-12 were applied to the C17.15 surface, and the binding of murine IL-12 to the immobilized C17.15 was measured as a function of time. Apparent dissociation and association rate constants were calculated, assuming a zero order dissociation and first order association kinetics as well as a simple one to one molecular interaction between the immobilized C17.15 and murine IL-12. From these measurements, the apparent dissociation (k_d, off-rate) and association (k_a, on-rate) rate constants were calculated. These results were used to calculate a K_d value for the interaction. An on-rate of 3.8 x 10⁵ M⁻¹s⁻¹, an off-rate of 1.84 x 10⁻⁴ s⁻¹, and a K_d of 4.8 x 10⁻¹⁰ was observed for the recombinant murine IL-12-C17.15 interaction.

- 25 The observed activities of C17.15 in neutralizing murine IL-12 activity and binding to cell surface receptors, as well as the kinetics of binding of C17.15 to murine IL-12 correlate with similar measurements for the J695-rhIL-12 interaction. This indicates that the modes of action of the rat anti-mouse IL-12 antibody C17.15 and anti-human IL-12 antibody J695 are nearly identical based upon on-rate, off-rate, K_d, IC₅₀, and the PHA blast assay. Therefore, C17.15 was used as a homologous antibody to J695 in murine models of inflammation and autoimmune disease to study the effects of IL-12 blockade on the initiation or progression of disease in these model animals (see Example 8).

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EXAMPLE 8: Treatment of Autoimmune or Inflammation-Based Diseases in Mice by α -Murine IL-12 Antibody Administration

A. Suppression of Collagen-Induced Arthritis in Mice by the α -IL-12 antibody C17.15

A correlation between IL-12 levels and rheumatoid arthritis (RA) has been demonstrated. For example, elevated levels of IL-12 p70 have been detected in the synovia of RA patients compared with healthy controls (Morita et al (1998) *Arthritis and Rheumatism*. 41: 306-314). Therefore, the ability of C17.15, a rat anti-mouse IL-12 antibody, to suppress collagen-induced arthritis in mice was assessed.

Male DBA/1 mice (10/group) were immunized with type II collagen on Day 0 and treated with C17.15, or control rat IgG, at 10 mg/kg intraperitoneally on alternate days from Day -1 (1 day prior to collagen immunization) to Day 12. The animals were monitored clinically for the development of arthritis in the paws until Day 90. The arthritis was graded as: 0- normal; 1- arthritis localized to one joint; 2- more than one joint involved but not whole paw; 3- whole paw involved; 4- deformity of paw; 5- ankylosis of involved joints. The arthritis score of a mouse was the sum of the arthritic grades in each individual paw of the mouse (max = 20). The results are expressed as mean \pm SEM in each group.

The results, as shown in Figure 4, indicate that an arthritic score was measurable in the C17.15-treated mice only after day 50 post-treatment, and that the peak mean arthritic score obtained with the C17.15-treated mice was at least 5-fold lower than that measured in the IgG-treated mice. This demonstrated that the rat anti-mouse IL-12 antibody C17.15 prevented the development of collagen-induced arthritis in mice.

B. Suppression of Colitis in Mice by the Rat α -Murine IL-12 Antibody C17.15

IL-12 has also been demonstrated to play a role in the development/pathology of colitis. For example, anti-IL-12 antibodies have been shown to suppress disease in mouse models of colitis, e.g., TNBS induced colitis IL-2 knockout mice (Simpson et al. (1998) *J. Exp. Med.* 187(8): 1225-34). Similarly, anti-IL-2 antibodies have been demonstrated to suppress colitis formation in IL-10 knock-out mice. The ability of the rat anti-mouse IL-12 antibody, C17.15, to suppress TNBS colitis in mice was assessed in two studies (Davidson et al. (1998) *J. Immunol.* 161(6): 3143-9).

In the first study, colitis was induced in pathogen free SJL mice by the administration of a 150 μ L 50% ethanol solution containing 2.0 mg TNBS delivered via a pediatric umbilical artery catheter into the rectum. Control animals were treated with a 150 μ L 50% ethanol solution only. A single dose of 0.75, 0.5, 0.25, or 0.1 mg C17.15 or 0.75 mg control rat IgG2a was given intravenously via the tail vein at day 11, and the therapeutic effect of the treatment was assessed by weighing the animals on days 11 and 17, and histological scoring at day 17. The weight of the mice treated with C17.15

increased within 48 hours of antibody treatment and normalized on day 6 after treatment. The effect of treatment with C17.15 was confirmed histologically. Further, assessments of IFN- γ secretion by CD4⁺ T-cells from spleen and colon of the treated mice, as well as IL-12 levels from spleen or colon-derived macrophages from the treated mice were also made (see Table 12).

In the second study, the dosing was optimized and the mice were treated with a total dose of 0.1 mg or 0.5 mg C17.15 or 0.1 mg control IgG2a, respectively, split between days 12 and 14. It was found that the administration of C17.15 in a single dose at the dosage of 0.1 mg/mouse or 0.25 mg/mouse led to only partial improvement in TNBS-induced colitis and did not result in a significant reduction in the CD4⁺ T cell production of IFN- γ *in vitro*, but did result in a significant decrease in secretion of IL-12, compared to untreated controls. At a single dose of 0.5 mg/mouse or greater a response was observed. Taking the lowest dose of antibody tested and administering it in two divided injections (at days 12 and 14) improved the dosing regimen, indicating that multiple low doses can be more effective than a single bolus dose. The data obtained are shown in Table 12.

Table 12: Anti-mouse IL-12 mAb C17.15 Suppresses Established Colitis in Mice

Disease Induction Day 0	Treatment Day 11	Weight (g)		IFN- γ spleen CD4 ⁺ cells (U/mL)	IL-12 spleen macrophages (pg/ml)
		Day 11	Day 17		
TNBS + Ethanol	Control IgG2a 0.75 mg	16.0	15.26	3326	300
TNBS + Ethanol	C17.15 0.75 mg	16.0	20.21	1732	0
TNBS + Ethanol	C17.15 0.5 mg	16.36	19.94	1723	0
TNBS + Ethanol	C17.15 0.25 mg	16.28	17.7	3618	7
TNBS + Ethanol	C17.15 0.1 mg	16.2	17.98	3489	22
Ethanol control	-	20.76	21.16	1135	0

20

Administration of C17.15 monoclonal anti-IL-12 in two divided doses spaced one day apart totaling 0.1 mg/mouse or 0.05 mg/mouse led to complete reversal of colitis as assessed by wasting and macroscopic appearance of the colon. In addition, this dose schedule led to significant down-regulation of lamina propria T-cell production of IFN- γ and macrophage production of IL-12, so that the latter were comparable to levels seen in control ethanol-treated mice without TNBS-colitis. Thus, C17.15 administration to mouse models for TNBS colitis reversed the progression of the disease in a dose-dependent manner.

25

C. Suppression of Experimental Autoimmune Encephalomyelitis (EAE) in Mice by α -IL-12 Antibodies

It is commonly believed that IL-12 plays a role in the pathogenesis of multiple sclerosis (MS). The inducible IL-12 p40 message has been shown to be expressed in acute plaques of MS patients but not in inflammatory brain infarct lesions (Windhagen, A. *et al.* (1995) *J. Exp. Med.* 182: 1985-1996). T cells from MS patients (but not control T cells) stimulate IL-12 production from antigen-presenting cells through unregulated CD40L expression (Balashov, K.E. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94: 599-603). MS patients have enhanced IFN- γ secretion that can be blocked with α -IL-12 antibodies *in vitro* (Balashov, K.E. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94: 599-603). Elevated levels of serum IL-12 are detected in MS patients, but not in other neurological diseases (Nicoletti, F. *et al.* (1996) *J. Neuroimmunol.* 70: 87-90). Increased IL-12 production has been shown to correlate with disease activity in MS patients (Cormabella, M. *et al.* (1998) *J. Clin. Invest.* 102: 671-678). The role of IL-12 in the pathogenesis of a murine model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), has been studied (Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181: 281-386; Banerjee, S. *et al.* (1998) *Arthritis Rheum.* (1998) 41: S33; and Segal, B.M. *et al.* (1998) *J. Exp. Med.* 187: 537-546). The disease in this model is known to be induced by T cells of the TH₁ subset. Therefore, the ability of α -IL-12 antibodies to prevent the onset of acute EAE was assessed.

An α -IL-12 antibody was found to be able to inhibit the onset of acute EAE, to suppress the disease after onset, and to decrease the severity of relapses in mice immunized with the autoantigen, myelin basic protein (Banerjee, S. *et al.* (1998) *Arthritis Rheum.* (1998) 41: S33). The beneficial effects of α -IL-12 antibody treatment in the mice persisted for over two months after stopping treatment. It has also been demonstrated that anti-IL-12 antibodies suppress the disease in mice that are recipients of encephalitogenic T cells by adoptive transfer (Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181: 281-386).

30 **EXAMPLE 9: Clinical Pharmacology of J695**

In a double blind, crossover study, 64 healthy, human male subjects were administered ascending doses of J695 or placebo. Measurement of complement fragment C3a prior to and 0.25 h after dosing did not demonstrate activation of the complement system. CRP and fibrinogen levels were only increased in subjects in whom symptoms of concurrent infections were observed.

All subjects survived and the overall tolerability of J695 was very good. In no case did treatment have to be stopped because of adverse events (AEs). The most commonly observed AEs were headache and common cold/bronchitis, neither of which
5 were categorized as severe.

One of the study subjects, a 33-year-old single male, was suffering from psoriasis guttata at the start of the study. According to the randomized study design, this subject by chance received 5mg/kg J695 by SC administration. Ten days prior to administration of the antibody, the subject showed only small discrete papular lesions on
10 the arms and legs. At the time of the antibody administration, the subject displayed increased reddening, thickness of the erythematous plaques, and increased hyperkeratosis. One week after J695 administration, the subject reported an improvement in skin condition, including flattening of the lesions and a decrease in scaling. Shortly after the second administration of J695 (5 mg/kg IV), the subject's skin
15 was totally cleared of psoriatic lesions, in the absence of any local treatment. Erythematous plaques covered with white scales reappeared concomitant with the expected clearance of J695 after the second administration of antibody.

EXAMPLE 10: Comparison of J695 Produced by Two CHO Cell Lines

20 For recombinant expression of J695, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells (Urlaub, G. and Chasin, L.A. (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220) by calcium phosphate-mediated transfection. Within the recombinant expression vector,
25 the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows
30 for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification.

One hundred and fifty micrograms of an expression vector encoding the peptide sequences of the human antibody J695 were dissolved in 2.7 ml water in a 50 ml conical tube. Three hundred μ L of 2.5 M CaCl_2 were added and this DNA mixture was
35 added dropwise to 3 ml of 2 x HEPES buffered saline in a 50 ml conical tube. After vortexing for 5 sec and incubating at room temperature for 20 min, 1 mL was distributed evenly over each plate (still in F12 medium), and the plates were incubated at 37 °C for 4 h. Liquid was removed by aspiration and 2 ml of 10% DMSO in F12 were added to

each plate. The DMSO shock continued for 1 min, after which the DMSO was diluted by the addition of 5 ml PBS to each plate. Plates were washed twice in PBS, followed by the addition of 10 ml of alpha MEM, supplemented with H/T and 5% FBS (selective for cells expressing DHFR) and overnight incubation at 37 °C. Cells were seeded into
 5 96-well plates at a density of 100 cells per well, and plates were incubated at 37 °C, 5% CO₂ for two weeks, with one change of medium per week.

Five days after the final medium change, culture supernatants were diluted 1:50 and tested using an ELISA specific for human IgG gamma chain. The clones yielding the highest ELISA signal were transferred from the 96-well plates to 12-well plates in
 10 1.5 ml/well of Alpha MEM + 5% dialyzed serum. After 3 days, another ELISA specific for human IgG gamma chain was performed, and the 12 clones with the greatest activity were split into the alpha MEM + 5% dialyzed serum and 20 nM MTX. Cell line 031898 218 grew in the presence of 20 nM MTX without any apparent cell death or reduction in growth rate, produced 1.8 µg/ml hIgG in a three-day assay. T-25 cultures of 031898
 15 218, growing in medium containing MTX, produced an average of 11.9 µg/ml of J695. The line, designated ALP903, was adapted to growth in suspension under serum-free conditions, where it produced 7.5 pg J695/cell/24h.

ALP903 cells, after initial selection in alpha MEM/5% FBS/20 nM MTX medium, were passed again in 20 nM MTX. The cells were cultured under 100 nM
 20 MTX selection, followed by passaging in 500 nM MTX twice in the next 30 days. At that time the culture was producing 32 µg J695/mL/24 h. The culture was subcloned by limiting dilution. Subclone 218-22 produced 16.5 µg/mL in a 96-well plate in 2 days and 50.3 µg/mL of J695 in a 12-well dish in 2 days. Clone 218-22 was cultured in alpha MEM/5% dialyzed FBS/500 nM MTX for 38 days, followed by adaptation to serum-
 25 free spinner culture, as above. The average cell-specific productivity of the serum-free suspension culture, designated ALP 905, was 58 pg/cell/24h.

The first cell line used to produce J695 (ALP 903) resulted in lower yields of the antibody from culture than a second cell line, ALP 905. To assure that the ALP 905-
 30 produced J695 was functionally identical to that produced from ALP 903, both batches of antibodies were assessed for IL-12 affinity, for the ability to block IL-12 binding to cellular receptors, for the ability to inhibit IFN-γ induction by IL-12, and for the ability to inhibit IL-12-mediated PHA blast proliferation.

The affinities of J695 batches ALP 903 and ALP 905 for IL-12 were determined by measuring the kinetic rate constants of binding to IL-12 by surface plasmon
 35 resonance studies (BIAcore analyses). The off-rate constant (k_d) and the on-rate constant (k_a) of antibody batches ALP903 and ALP905 for binding to rhIL-12 were determined in three experiments (as described in Example 3). The affinity, K_d , of binding to IL-12 was calculated by dividing the off-rate constant by the on-rate constant.

K_d was calculated for each separate experiment and then averaged. The results showed that the determined kinetic parameters and affinity of binding to rhIL-12 were very similar for J695 batches ALP 903 and ALP 905: the calculated K_d was $1.19 \pm 0.22 \times 10^{-10}$ M for batch ALP 903 and $1.49 \pm 0.47 \times 10^{-10}$ M for batch ALP 905 (see Table 13).

5 The ability of J695 derived from both ALP 903 and ALP 905 to block binding of rhIL-12 to IL-12 receptors on human PHA-activated T-lymphoblasts was assessed (see Example 3). Each sample of J695 was tested at a starting concentration of 1×10^{-8} with 10-fold serial dilutions. The antibody was preincubated for 1 hour at 37 °C with 50 pM [125 I]-human IL-12 in binding buffer. PHA blast cells were added to the antibody/[125 I]-
10 human IL-12 mixture and incubated for 2 h at room temperature. Cell bound radioactivity was separated from free [125 I]-IL-12 by centrifugation and washing steps, and % inhibition was calculated. The IC_{50} values for J695 were determined from the inhibition curves using 4-parameter curve fitting and were confirmed by two independent experiments. Incubations were carried out in duplicate. The results for the
15 two batches of J695 were very similar (see Table 13).

The ability of J695 from both ALP 903 and ALP 905 cells to inhibit rhIL-12-induced IFN- γ production by human PHA-activated lymphoblasts *in vitro* was assessed. Serial dilutions of J695 were preincubated with 200 pg/mL rhIL-12 for 1 h at 37 °C. PHA lymphoblast cells were added and incubated for 18 hours at 37 °C. After
20 incubation, cell free supernatant was withdrawn and the level of human IFN- γ determined by ELISA. The IC_{50} values from the inhibition curves were plotted against the antibody concentration using 4-parameter curve fitting. The results demonstrate that the ability of the two batches to inhibit IFN- γ production is very similar.

The *in vitro* PHA blast cell proliferation assay was used to measure the
25 neutralization capacity of ALP 903 and ALP 905 J695 for rhIL-12. Serial dilutions of J695 of each type were preincubated with 230 pg/mL human IL-12 for 1 h at 37 °C. Next PHA blast cells were added and incubated for 3 days at 37 °C. The cells were then labeled for 6 hours with 1 γ Ci/well [3 H]-thymidine. The cultures were harvested and [3 H]-thymidine incorporation measured. Non-specific proliferation (background) was
30 measured in the absence of rhIL-12. The IC_{50} values for ALP 903 and ALP 905 J695 were found to be very similar and are set forth in Table 13.

The activity of the J695 antibodies in neutralizing rhIL-12 activity, in blocking IL-12 binding to cell surface receptors, and in binding to rhIL-12 did not significantly differ from batch ALP 903 to batch ALP 905, and thus the antibodies produced from
35 these two different cell types were equivalent.

Table 13: Comparison of the Properties of J695 lots ALP 903 and ALP 905

Antibody	k_a , On-rate (M^{-1}, s^{-1})	k_d , Off-rate (s^{-1})	K_d (M)	RB assay IC_{50} (M)	PHA blast Assay IC_{50} (M)	IFN- γ Assay IC_{50} (M)
J695 ALP 903	3.75×10^5	4.46×10^{-5}	1.19×10^{-10}	3.4×10^{-11}	5.5×10^{-12}	5.8×10^{-12}
J695 ALP 905	3.91×10^5	5.59×10^{-5}	1.49×10^{-10}	3.0×10^{-11}	4.4×10^{-12}	4.3×10^{-12}

5 EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. An isolated human antibody, or an antigen-binding portion thereof, that binds to human IL-12, wherein said human antibody is a neutralizing antibody.

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2. A selectively mutated human IL-12 antibody, comprising:
a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis, contact or hypermutation position with an activity enhancing amino acid residue such that it binds to human IL-12.

10

3. A selectively mutated human IL-12 antibody, comprising:
a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis position with an activity enhancing amino acid residue such that it binds to human IL-12.

15

4. The selectively mutated human IL-12 antibody of claim 2, wherein the human antibody or antigen-binding portion thereof is selectively mutated at more than one preferred selective mutagenesis, contact or hypermutation positions with an activity enhancing amino acid residue.

20

5. The selectively mutated human IL-12 antibody of claim 4, wherein the human antibody or antigen-binding portion thereof is selectively mutated at no more than three preferred selective mutagenesis, contact or hypermutation positions.

25

6. The selectively mutated human IL-12 antibody of claim 4, wherein the human antibody or antigen-binding portion thereof is selectively mutated at no more than two preferred selective mutagenesis, contact or hypermutation positions.

7. The selectively mutated human IL-12 antibody of claim 2, wherein the human antibody or antigen-binding portion thereof, is selectively mutated such that a target specificity affinity level is attained, said target level being improved over that attainable when selecting for an antibody against the same antigen using phage display technology.

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8. The selectively mutated human IL-12 antibody of claim 7, wherein the selectively mutated human antibody further retains at least one desirable property or characteristic.

9. An isolated human antibody, or antigen-binding portion thereof, that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6}\text{M}$ or less.

10. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-2}\text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-7}\text{M}$ or less.

11. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-3}\text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-8}\text{M}$ or less.

12. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-4}\text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-9}\text{M}$ or less.

13. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-5}\text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-10}\text{M}$ or less.

14. The isolated human antibody of claim 9, or an antigen-binding portion thereof, which dissociates from human IL-12 with a k_{off} rate constant of $1 \times 10^{-5}\text{s}^{-1}$ or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-11}\text{M}$ or less.

15. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-6}\text{M}$ or less;

b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1; and

c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2.

16. The isolated human antibody of claim 15, or an antigen-binding portion thereof, which further has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4.

17. The isolated human antibody of claim 15, or an antigen-binding portion thereof, which further has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6.

18. The isolated human antibody, or antigen binding portion thereof of claim 16, which has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and has a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

19. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-9}M$ or less;

b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9; and

c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

20. The isolated human antibody of claim 19, or an antigen-binding portion thereof, which further has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12.

21. The isolated human antibody of claim 19, or an antigen-binding portion thereof, which further has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

22. The isolated human antibody of claim 19, which has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15; and has a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

- 5 23. An isolated human antibody, or an antigen-binding portion thereof, which
- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1×10^{-9} M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and
- 10 c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18.

24. The isolated human antibody, or an antigen-binding portion thereof, of claim 23 which further has a heavy chain CDR2 comprising the amino acid sequence of

15 SEQ ID NO: 19; and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20.

25. The isolated human antibody, or an antigen-binding portion thereof, of claim 23 which further has a heavy chain CDR1 comprising the amino acid sequence of

20 SEQ ID NO: 21; and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.

26. An isolated human antibody, or an antigen-binding portion thereof, having a heavy chain variable region comprising the amino acid sequence of SEQ ID

25 NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

27. The isolated human antibody of claim 26, comprising a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA

30 and IgE constant regions.

28. The isolated human antibody of claim 27, wherein the antibody heavy chain constant region is IgG1.

35 29. The isolated human antibody of claim 26, which is a Fab fragment.

30. The isolated human antibody of claim 26, which is a F(ab')₂ fragment.

31. The isolated human antibody of claim 26, which is a single chain Fv fragment.

32. An isolated human antibody, or an antigen-binding portion thereof, which
- 5 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 404-SEQ ID NO: 469; or
- c) has a light chain CDR3 comprising the amino acid sequence selected
- 10 from the group consisting of SEQ ID NO: 534-SEQ ID NO: 579.

33. The isolated human antibody, or an antigen-binding portion thereof, of claim 32 which further has a heavy chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:335-SEQ ID NO: 403; or a light chain

15 CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 506-SEQ ID NO: 533.

34. The isolated human antibody, or an antigen-binding portion thereof, of claim 32 which further has a heavy chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 288-SEQ ID NO: 334; or a light

20 chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 470-SEQ ID NO: 505.

35. An isolated human antibody, or an antigen-binding portion thereof,

25 having a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

36. The isolated human antibody of claim 35, comprising a heavy chain

30 constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions.

37. The isolated human antibody of claim 36, wherein the antibody heavy chain constant region is IgG1.

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38. The isolated human antibody of claim 35, which is a Fab fragment.

39 The isolated human antibody of claim 35, which is a F(ab')₂ fragment.

40. The isolated human antibody of claim 35, which is a single chain Fv fragment.

- 5 41. An isolated human antibody, or an antigen-binding portion thereof, which
- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25; and
- 10 c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

 42. The isolated human antibody, or an antigen-binding portion thereof, of claim 41 which further has a heavy chain CDR2 comprising the amino acid sequence of

15 SEQ ID NO: 27; and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28.

 43. The isolated human antibody, or an antigen-binding portion thereof, of claim 41 which further has a heavy chain CDR1 comprising the amino acid sequence of

20 SEQ ID NO: 29; and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

 44. An isolated human antibody, or an antigen-binding portion thereof, having a heavy chain variable region comprising the amino acid sequence of SEQ ID

25 NO: 31, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 32.

 45. The isolated human antibody of claim 44, comprising a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA

30 and IgE constant regions.

 46. The isolated human antibody of claim 45, wherein the antibody heavy chain constant region is IgG1.

35 47. The isolated human antibody of claim 44, which is a Fab fragment.

 48. The isolated human antibody of claim 44, which is a F(ab')₂ fragment.

49. The isolated human antibody of claim 44, which is a single chain Fv fragment.

50. An isolated human antibody, or an antigen-binding portion thereof, which
5 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁶M or less;

b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5,
10 or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a heavy chain CDR1 comprising the amino acid sequence of
15 SEQ ID NO: 5; and

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a mutant thereof having one or more amino acid substitutions at a contact position or a
20 hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6.

25 51. An isolated human antibody, or an antigen-binding portion thereof, which
a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC₅₀ of 1 x 10⁻⁹M or less;

b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold
30 higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a heavy chain CDR1 comprising the amino acid sequence of
35 SEQ ID NO: 13; and

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID

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NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

52. An isolated human antibody, or an antigen-binding portion thereof, which
10 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC_{50} of $1 \times 10^{-9}\text{M}$ or less;

b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21; and
15 20

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.
25 30

53. An isolated nucleic acid encoding the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17.

54. The isolated nucleic acid of claim 53, which encodes an antibody heavy chain variable region.
35

55. The isolated nucleic acid of claim 54, wherein the CDR2 of the antibody heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 19.

56. The isolated nucleic acid of claim 54, wherein the CDR1 of the antibody heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 21.

5 57. The isolated nucleic acid of claim 56, which encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23.

58. An isolated nucleic acid encoding the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18.

10

59. The isolated nucleic acid of claim 58, which encodes an antibody light chain variable region.

60. The isolated nucleic acid of claim 59, wherein the CDR2 of the antibody
15 light chain variable region comprises the amino acid sequence of SEQ ID NO: 20.

61. The isolated nucleic acid of claim 59, wherein the CDR1 of the antibody light chain variable region comprises the amino acid sequence of SEQ ID NO: 22.

62. The isolated nucleic acid of claim 61, which encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

63. An isolated human antibody, or an antigen-binding portion thereof, which

a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay

25 with an IC₅₀ of 1 x 10⁻⁹M or less;

b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and

c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a mutant thereof having one or more amino acid substitutions at a contact position

or a hypermutation position, wherein said mutant has a k_{off} rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.

64. An isolated nucleic acid encoding the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25.

65. The isolated nucleic acid of claim 64, which encodes an antibody heavy chain variable region.

66. The isolated nucleic acid of claim 65, wherein the CDR2 of the antibody heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 27.

67. The isolated nucleic acid of claim 65, wherein the CDR1 of the antibody heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 29.

68. The isolated nucleic acid of claim 67, which encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31.

69. An isolated nucleic acid encoding the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

70. The isolated nucleic acid of claim 69, which encodes an antibody light chain variable region.

71. The isolated nucleic acid of claim 70, wherein the CDR2 of the antibody light chain variable region comprises the amino acid sequence of SEQ ID NO: 28.

72. The isolated nucleic acid of claim 70, wherein the CDR1 of the antibody light chain variable region comprises the amino acid sequence of SEQ ID NO: 30.

73. The isolated nucleic acid of claim 72, which encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 32.

74. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

5 b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the VH3 germline family, wherein the heavy chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

10 c) has a light chain variable region comprising an amino acid sequence selected from a member of the V λ 1 germline family, wherein the light chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

75. An isolated human antibody, or an antigen-binding portion thereof, which
15 has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

20 b) has a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 595-667, wherein the heavy chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

25 c) has a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 669-675, wherein the light chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

76. An isolated human antibody, or an antigen-binding portion thereof, which
30 has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

35 b) has a heavy chain variable region comprising the COS-3 germline amino acid sequence, wherein the heavy chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

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c) has a light chain variable region comprising the DPL8 germline amino acid sequence, wherein the light chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

5 77. An isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

 a) that binds to human IL-12 and dissociates from human IL-12 with a k_{off} rate constant of 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast
10 proliferation assay (PHA assay) with an IC_{50} of $1 \times 10^{-6} \text{ M}$ or less.

 b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the VH3 germline family, wherein the heavy chain variable region comprises a CDR2 that is structurally similar to CDR2s from other VH3 germline family members, and a CDR1 that is structurally similar to CDR1s from other VH3
15 germline family members, and wherein the heavy chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue;

 c) has a light chain variable region comprising an amino acid sequence selected from a member of the V λ 1 germline family, wherein the light chain variable region comprises a CDR2 that is structurally similar to CDR2s from other V λ 1 germline
20 family members, and a CDR1 that is structurally similar to CDR1s from other V λ 1 germline family members, and wherein the light chain variable region has a mutation at a contact or hypermutation position with an activity enhancing amino acid residue.

 78 The isolated human antibody, or antigen binding portion thereof, of claim
25 74, wherein the mutation is in the heavy chain CDR3.

 79. The isolated human antibody, or antigen binding portion thereof, of claim 74, wherein the mutation is in the light chain CDR3.

30 80. The isolated human antibody, or antigen binding portion thereof, of claim 74, wherein the mutation is in the heavy chain CDR2.

 81. The isolated human antibody, or antigen binding portion thereof, of claim 74, wherein the mutation is in the light chain CDR2.

35 82. The isolated human antibody, or antigen binding portion thereof, of claim 74, wherein the mutation is in the heavy chain CDR1.

83. The isolated human antibody, or antigen binding portion thereof, of claim 74, wherein the mutation is in the light chain CDR1.

84. A recombinant expression vector encoding:

5 a) an antibody heavy chain having a variable region comprising the amino acid sequence of SEQ ID NO: 31; and

b) an antibody light chain having a variable region comprising the amino acid sequence of SEQ ID NO: 32.

10 85. A host cell into which the recombinant expression vector of claim 84 has been introduced.

86. A method of synthesizing a human antibody that binds human IL-12, comprising culturing the host cell of claim 85 in a culture medium until a human
15 antibody that binds human IL-12 is synthesized by the cell.

87. An isolated human antibody, or antigen-binding portion thereof, that neutralizes the activity of human IL-12, and at least one additional primate IL-12 selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-
20 12, cynomolgus IL-12 and rhesus IL-12, but which does not neutralize the activity of the mouse IL-12.

88. A pharmaceutical composition comprising the antibody or an antigen binding portion thereof, of claim 1 and a pharmaceutically acceptable carrier.
25

89. A composition comprising the antibody or an antigen binding position thereof, of claim 1 and an additional agent.

90. The composition of claim 89, wherein the additional agent is a
30 therapeutic agent.

91. The composition of claim 90, wherein the therapeutic agent is selected from the group consisting of budenoside, epidermal growth factor, corticosteroids, cyclosporin, sulfasalazine, aminosalicylates, 6-mercaptopurine, azathioprine, metronidazole, lipoxxygenase inhibitors, mesalamine, olsalazine, balsalazide, antioxidants, thromboxane inhibitors, IL-1 receptor antagonists, anti-IL-1 β monoclonal antibodies, anti-IL-6 monoclonal antibodies, growth factors, elastase inhibitors, pyridinyl-imidazole compounds, antibodies or agonists of TNF, LT, IL-1, IL-2, IL-6, IL-
35

7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF, antibodies of CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands, methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, ibuprofen, corticosteroids, prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, IRAK, NIK, IKK, p38, MAP kinase inhibitors, IL-1 β converting enzyme inhibitors, TNF α converting enzyme inhibitors, T-cell signalling inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors, soluble p55 TNF receptor, soluble p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R, antiinflammatory cytokines, IL-4, IL-10, IL-11, IL-13 and TGF β .

92. The therapeutic composition of claim 90, wherein the therapeutic agent is selected from the group consisting of anti-TNF antibodies, and antibody fragments thereof, TNFR-Ig constructs, TACE inhibitors, PDE4 inhibitors, corticosteroids, budenoside, dexamethasone, sulfasalazine, 5-aminosalicylic acid, olsalazine, IL-1 β converting enzyme inhibitors, IL-1ra, tyrosine kinase inhibitors, 6-mercaptopurines and IL-11.

93. The therapeutic composition of claim 90, wherein the therapeutic agent is selected from the group consisting of corticosteroids, prednisolone, methylprednisolone, azathioprine, cyclophosphamide, cyclosporine, methotrexate, 4-aminopyridine, tizanidine, interferon- β 1a, interferon- β 1b, Copolymer 1, hyperbaric oxygen, intravenous immunoglobulin, clabribine, antibodies or agonists of TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, PDGF, antibodies to CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands, methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, ibuprofen, corticosteroids, prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, IRAK, NIK, IKK, p38 or MAP kinase inhibitors, IL-1 β converting enzyme inhibitors, TACE inhibitors, T-cell signalling inhibitors, kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors, soluble p55 TNF receptor, soluble p75 TNF receptor, sIL-1RI, sIL-1RII, sIL-6R, sIL-13R, anti-P7s, p-selectin glycoprotein ligand (PSGL), antiinflammatory cytokines, IL-4, IL-10, IL-13 and TGF β .

94. A method for inhibiting human IL-12 activity comprising contacting human IL-12 with the antibody of claim 44 such that human IL-12 activity is inhibited.

95. A method for inhibiting human IL-12 activity in a human subject suffering from a disorder in which IL-12 activity is detrimental, comprising administering to the human subject the antibody of claim 44 such that human IL-12 activity in the human subject is inhibited.

96. The method of claim 95, wherein the disorder is selected from the group consisting of rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, ankylosing spondylitis, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, multiple sclerosis, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis, scleroderma, thyroiditis, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, polyarteritis nodosa, Wegener's granulomatosis, Henoch-Schonlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, Sjogren's syndrome, uveitis, sepsis, septic shock, sepsis syndrome, adult respiratory distress syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, myasthenia gravis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, fibrotic lung diseases, hemolytic anemia, malignancies, heart failure and myocardial infarction.

97. The method of claim 95, wherein the disorder is Crohn's disease.

98. The method of claim 95, wherein the disorder is multiple sclerosis.

99. The method of claim 95, wherein the disorder is rheumatoid arthritis.

100. A method for improving the activity of an antibody, or antigen-binding portion thereof, to attain a predetermined target activity, comprising:

- a) providing a parent antibody a antigen-binding portion thereof;
- b) selecting a preferred selective mutagenesis position selected from group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.

c) individually mutating the selected preferred selective mutagenesis position to at least two other amino acid residues to hereby create a first panel of mutated antibodies, or antigen binding portions thereof;

d) evaluating the activity of the first panel of mutated antibodies, or antigen binding portions thereof to determined if mutation of a single selective mutagenesis position produces an antibody or antigen binding portion thereof with the predetermined target activity or a partial target activity;

e) combining in a stepwise fashion, in the parent antibody, or antigen binding portion thereof, individual mutations shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof.

f) evaluating the activity of the combination antibodies, or antigen binding portions thereof to determined if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity.

g) if steps d) or f) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result an antibody with only a partial activity, the method further comprising mutating additional amino acid residues selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 to at least two other amino acid residues to thereby create a second panel of mutated antibodies or antigen-binding portions thereof;

h) evaluating the activity of the second panel of mutated antibodies or antigen binding portions thereof, to determined if mutation of a single amino acid residue selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 results an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

i) combining in stepwise fashion in the parent antibody, or antigen-binding portion thereof, individual mutations of step g) shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof;

j) evaluating the activity of the combination antibodies or antigen binding portions thereof, to determined if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity;

k) if steps h) or j) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result in an antibody with only a partial activity, the method further comprising mutating additional amino acid residues selected from the group consisting of H33B, H52B and L31A to at least two other amino acid residues to thereby create a third panel of mutated antibodies or antigen binding portions thereof;

l) evaluating the activity of the third panel of mutated antibodies or antigen binding portions thereof, to determine if a mutation of a single amino acid

residue selected from the group consisting of H33B, H52B and L31A resulted in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

m) combining in a stepwise fashion in the parent antibody, or antigen
5 binding portion thereof, individual mutation of step k) shown to have an improved activity, to form combination antibodies, or antigen binding portions, thereof;

n) evaluating the activity of the combination antibodies or antigen-
binding portions thereof, to determine if the combination antibodies, or antigen binding
portions thereof have the predetermined target activity to thereby produce an antibody or
10 antigen binding portion thereof with a predetermined target activity.

101. A method for improving the activity of an antibody, or antigen-binding
portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

15 b) selecting a preferred selective mutagenesis position, contact or hypermutation
position within a complementarity determining region (CDR) for mutation, thereby
identifying a selected preferred selective mutagenesis position, contact or hypermutation
position;

c) individually mutating said preferred selective mutagenesis position, contact or
20 hypermutation position to at least two other amino acid residues to thereby create a
panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding
portions thereof, relative to the parent antibody or antigen-binding portion thereof;

e) repeating steps b) through d) for at least one other preferred selective
25 mutagenesis position, contact or hypermutation position if the desired antibody activity
is not obtained;

f) combining in a stepwise fashion, in the parent antibody, or antigen-binding
portion thereof, individual mutations shown to have improved activity, to form
combination antibodies, or antigen-binding portions thereof; and

30 g) evaluating the activity of the combination antibodies, or antigen-binding
portions thereof, relative to the parent antibody or antigen-binding portion thereof;
until an antibody, or antigen-binding portion thereof, with an improved activity, relative
to the parent antibody, or antigen-binding portion thereof, is obtained.

35 102. The method of claim 101, wherein contact positions are selected from the
group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54,
H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91,
L92, L93, L94 and L96.

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103. The method of claim 101, wherein hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93.

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104. The method of claim 101, wherein the preferred positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.

10

105. The method of claim 101, wherein the contact positions are selected from the group consisting of L50 and L94.

106. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

15

a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity is not further improved by mutagenesis in said phage-display system;

b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;

e) repeating steps b) through d) for at least one other preferred selective mutagenesis position, contact or hypermutation position if the desired antibody activity is not obtained;

f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

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107. The method of claim 106, wherein contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96.

5

108. The method of claim 106, wherein hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93.

10

109. The method of claim 106, wherein preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94

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110. The method of claim 106, wherein the contact positions are selected from the group consisting of L50 and L94.

111. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

20

a) providing a recombinant parent antibody or antigen-binding portion thereof ;
b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

25

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expressing said panel in an appropriate expression system;

30

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

35

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for at least one other property or characteristic, wherein the property or characteristic is one that needs to be retained in the antibody;
until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

112. The method of claim 111, wherein contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins,
 5 preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

113. The method of claim 111, wherein the hypermutation positions are
 10 selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

114. The method of claim 111, wherein the preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-
 20 crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

115. The method of claim 111, wherein the contact positions are selected from
 25 the group consisting of L50 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

116. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- 35 b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

5 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one
10 other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) repeating steps a) through e) for at least one other preferred selective
15 mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

20 h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

25

117. The method of claim 116, wherein contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96, and wherein the other property or characteristic is selected from
30 the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

118. The method of claim 116, wherein the hypermutation positions are
35 selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation

of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

119. The method of claim 116 wherein the preferred selective mutagenesis
5 positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline
10 immunoglobulin sequence.

120. The method of claim 116, wherein the contact positions are selected from the group consisting of L50 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other
15 proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

121. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:
20 a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
b) selecting a contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or
25 hypermutation position;
c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;
30 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one
35 additional property or characteristic, wherein the property or characteristic is one that needs to be retained,

until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic relative to the parent antibody, or antigen-binding portion thereof, is obtained.

- 5 122. The method of claim 121, wherein contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins,
10 preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

123. The method of claim 121, wherein the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31,
15 L32, L53 and L93, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

- 20 124. The method of claim 121, wherein the contact positions are selected from the group consisting of L50 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

- 25 125. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be
30 further improved by mutagenesis in said phage-display system;
 b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position contact or hypermutation position;
35 c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) repeating steps a) through e) for at least one other preferred selective mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic relative to the parent antibody, or antigen-binding portion thereof, is obtained.

126. The method of claim 125, wherein contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

127. The method of claim 125, wherein the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

128. The method of claim 125 wherein the preferred selective mutagenesis positions are selected from the group consisting of H30, H31, H31B, H32, H33, H52,

H56, H58, L30, L31, L32, L50, L91, L92, L93 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

129. The method of claim 125, wherein the contact positions are selected from the group consisting of L50 and L94, and wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other proteins, preservation of non-crossreactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

130. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting a amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic;

until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

131. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
- b) selecting a amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other position within the CDR which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

132. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof, that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting a selecting an amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and;

c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic, until an antibody, or antigen-binding portion

thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

133. A method for improving the activity of an antibody, or antigen-binding
5 portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region
10 (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions
15 thereof and expression in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other position within the CDR
20 which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 ;

f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity,
25 to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and other property or characteristics of the combination antibodies, or antigen-binding portions thereof, with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof;
until an antibody, or antigen-binding portion thereof, with an improved activity, relative
30 to the parent antibody, or antigen-binding portion thereof, is obtained.

134. A method for improving the activity of an antibody, or antigen-binding portion thereof, without affecting other properties, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region
35 (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic, wherein the property or characteristic needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and retained property, or characteristic relative to the parent antibody, or antigen-binding portion thereof, is obtained.

135. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies or antigen-binding portions thereof, relative to the parent antibody or antigen-portion thereof, for changes in at least one other property or characteristic;

f) repeating steps b) through e) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity but not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof with at least one retained property or characteristic; and

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- h) evaluating the activity and the retention of at least one property of characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

136. A method to improve the affinity of an antibody or antigen-binding portion thereof, comprising:
- a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;
- d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
- e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

137. A method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:
- a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

5 d) evaluating the activity and retention of at least one other property or characteristic of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

10 e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

15 f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and not to affect at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

20 g) evaluating the activity and retention of at least one other property or characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one other retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

138. The method of claim 130, wherein the other property or characteristic is selected from the group consisting of preservation of non-crossreactivity with other
25 proteins, preservation of non-cross reactivity with other human tissues, preservation of epitope recognition and an antibody with a close to germline immunoglobulin sequence.

139. A method for detecting human IL-12 comprising contacting human IL-12 with the antibody, or antigen-binding portion thereof, of claim 1 such that human IL-12
30 is detected.

140. The method of claim 139, wherein human IL-12 is detected *in vitro*.

141. The method of claim 139, wherein human IL-12 is detected in a
35 biological sample for diagnostic purposes.

HUMAN ANTIBODIES THAT BIND HUMAN IL-12 AND METHODS FOR PRODUCING

Abstract

5

Human antibodies, preferably recombinant human antibodies, that specifically bind to human interleukin-12 (hIL-12) are disclosed. Preferred antibodies have high affinity for hIL-12 and neutralize hIL-12 activity *in vitro* and *in vivo*. An antibody of the invention can be a full-length antibody or an antigen-binding portion thereof. The antibodies, or antibody portions, of the invention are useful for detecting hIL-12 and for inhibiting hIL-12 activity, *e.g.*, in a human subject suffering from a disorder in which hIL-12 activity is detrimental. Nucleic acids, vectors and host cells for expressing the recombinant human antibodies of the invention, and methods of synthesizing the recombinant human antibodies, are also encompassed by the invention.

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Figure 1A. Heavy Chain Variable Region Sequences

SEQ ID NO:	Kabat number	CDR H1	CDR H2
33	JOE9wt VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
35	Cos-3/Jh3 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
37	70-1 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
39	78-34 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
41	79-1 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
43	101-11 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
45	26-1 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
47	136-15 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
49	136-15 VH germline	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
51	149-5 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
53	149-6 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
55	103-4 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
57	103-8 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
59	103-14 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
61	G6 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
63	Y139 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
65	AO3 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
67	AO3 VH germline	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
23	Y61 VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
69	Y61 VH germline	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
71	Y61-H31E VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
73	Y61 L50Y VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
75	Y61-L94Y VH	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN
31	J695	QVQLVQSGG	SVGMH WVRQAPGKGLEWVA FIRYDGSN

CDR H3

[illegible]

Figure 1C. Light Chain Variable Region Sequences

SEQ ID NO:	Kabat number	CDR L1	CDR L2
34	Joe9 VL wt	SYVLTQPPSVSGT	SGGRSNIGSNTVK
36	Dp18 Lv1042/Jr1	Q S A	T . S S A g r D . H
38	70-1 VL	Q S	Q S
40	78-34 VL	Q S	Q S
42	79-1 VL	Q S	Q S
44	101-11 VL	Q S	Q S
46	26-1 VL	Q S	Q S
48	136-15 VL	Q S	Q S
50	136-15 VL germline	Q S A	Q S S
52	149-5 VL	Q S	Q S
54	149-6 VL	Q S	Q S
56	103-4 VL	Q S	Q S
58	103-8 VL	Q S	Q S
60	103-14 VL	Q S	Q S
62	G6 VL	Q S	Q S
64	Y139 VL	Q S	Q S
66	AO3 VL	Q S	Q S
68	AO3 VL germline	Q S	Q S
24	Y61 VL	Q S	Q S
70	Y61 VL germline	Q S	Q S
72	Y61-H31E VL	Q S	Q S
74	Y61-L50Y VL	Q S	Q S
76	Y61-L94Y VL	Q S	Q S
32	J695 VL	Q S	Q S

Figure 1D. Light Chain Variable Region Sequences

SEQ ID No:	Kabat number		CDR L3
34	Joe9 VL wt	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
36	Dp18 Lv1042/JA1	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
38	70-1 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
40	78-34 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
42	79-1 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
44	101-11 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
46	26-1 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
48	136-15 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
50	136-15 VL germline	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
52	149-5 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
54	149-6 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
56	103-4 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
58	103-8 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
60	103-14 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
62	G6 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
64	Y139 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
66	AO3 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
68	AO3 VL germline	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
24	Y61 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
70	Y61 VL germline	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
72	Y61-H31E VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
74	Y61-L50Y VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
76	Y61-L94Y VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107
32	J695 VL	GV	555560126364556676869707172737475767778798081828384858687888990919293949596979899100101102103104105106107

Figure 2A. Y61 Heavy Chain CDR H1 Mutagenesis

SEQ ID NO:		CDR H1										k_{off} ($\times 10^5$)
		27	28	29	30	31	32	33	34	35		
21	Y61	F	T	F	S	S	Y	G	M	H		
288		.	.	.	E	22.8	
289		.	.	.	S	16.8	
290		.	.	.	Y	31.9	
291		.	.	.	H	29.6	
292		.	.	.	K	22.5	
293		.	.	.	R	24.5	
294		.	.	.	N	30.1	
295		.	.	.	T	32.0	
296		.	.	.	G	23.3	
297		.	.	.	V	39.9	
298		.	.	.	I	20.7	
299		.	.	.	W	21.6	
300		E	21.9	
301		C	12.0	
302		S	24.9	
303		Y	39.8	
304		H	30.9	
305		R	66.4	
306		N	19.1	
307		Q	15.2	
308		T	71.6	
309		A	20.5	
310		I	33.4	
311		E	.	.	.	229.0	
312		C	.	.	.	383.0	
313		S	.	.	.	157.5	
314		Y	.	.	.	33.7	
315		H	.	.	.	46.1	
316		R	.	.	.	448.5	
317		N	.	.	.	297.0	
318		T	.	.	.	148.0	
319		A	.	.	.	165.5	
320		V	.	.	.	133.5	
321		L	.	.	.	226.0	
322		I	.	.	.	160.5	
323		D	.	.	152.0	
324		E	.	.	189.0	
325		C	.	.	286.5	
326		S	.	.	39.9	
327		Y	.	.	250.5	
328		N	.	.	30.8	
329		G	.	.	17.8	
330		A	.	.	27.3	
331		V	.	.	191.0	
332		M	.	.	21.5	
333		I	.	.	250.0	
334		P	.	.	159.5	

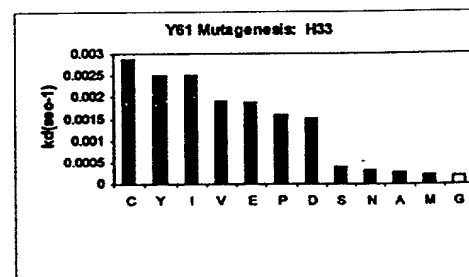
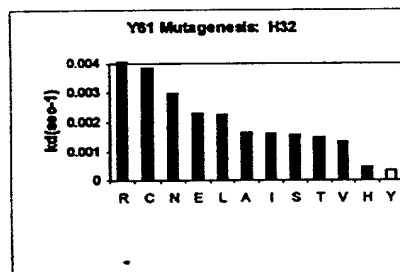
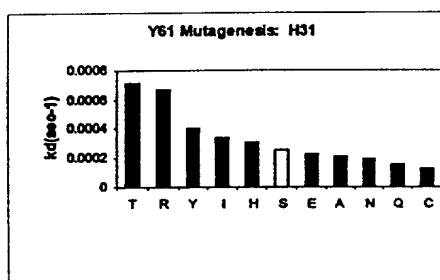
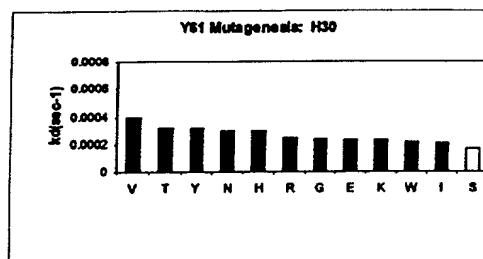


Figure 2B. Y61 Heavy Chain CDR H2 Mutagenesis

SEQ ID NO:		CDR H2																k_{off} ($\times 10^5$)
		50	51	52	52A	53	54	55	56	57	58	59	60	61	62	63	64	
19	Y61	F	I	R	Y	D	G	S	N	K	Y	Y	A	D	S	V	K	G
335		E	34.7
336		C	28.5
337		Y	23.0
338		H	30.9
339		K	61.2
340		N	34.4
341		Q	42.0
342		T	20.5
343		L	44.0
344		F	20.4
345		.	.	E	31.8
346		.	.	S	29.2
347		.	.	Y	29.8
348		.	.	H	40.7
349		.	.	K	26.2
350		.	.	R	20.6
351		.	.	Q	28.5
352		.	.	T	37.4
353		.	.	G	32.1
354		.	.	A	17.1
355		.	.	V	31.7
356		.	.	L	34.7
357		.	.	W	35.1
358		.	.	.	D	15.1
359		.	.	.	E	39.9
360		.	.	.	S	36.8
361		.	.	.	Y	61.1
362		.	.	.	K	158.0
363		.	.	.	R	166.5
364		.	.	.	N	72.7
365		.	.	.	Q	79.2
366		.	.	.	T	50.0
367		.	.	.	A	40.4
368		.	.	.	V	44.0
369		.	.	.	L	109.5
370		.	.	.	I	94.4
371		.	.	.	F	168.5
372		.	.	.	D	45.5
373		.	.	.	E	35.1
374		.	.	.	S	37.3
375		.	.	.	Y	64.6
376		.	.	.	K	40.7
377		.	.	.	R	2.5
378		.	.	.	N	44.7
379		.	.	.	Q	31.6
380		.	.	.	T	64.4
381		.	.	.	G	17.8
382		.	.	.	V	43.5

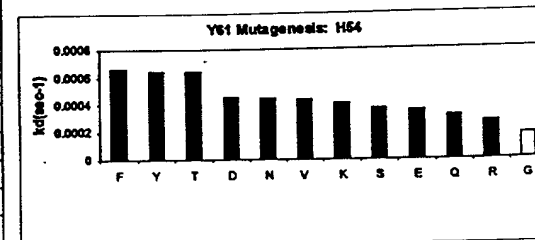
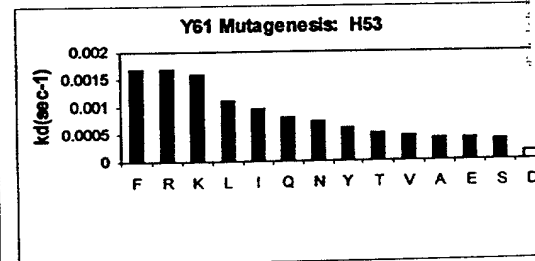
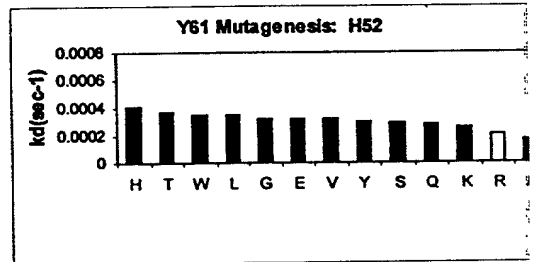
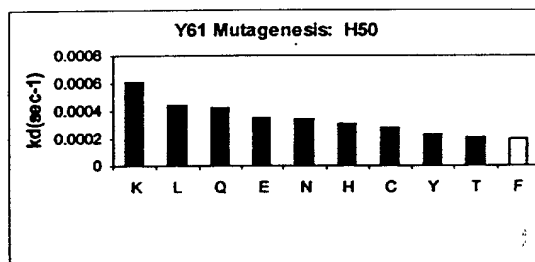


Figure 2C. Y61 Heavy Chain CDR H2 Mutagenesis

		CDR H2																	
SEQ ID NO:		50	51	52	52A	53	54	55	56	57	58	59	60	61	62	63	64	65	k_{off} ($\times 10^5$)
19	Y61	F	I	R	Y	D	G	S	N	K	Y	Y	A	D	S	V	K	G	
383		F	.	S	66.3
384		S	62.4
385		Y	39.0
386		H	42.0
387		N	38.5
388		T	23.5
389		G	27.2
390		M	38.3
391		L	26.4
392		I	16.9
393		P	29.9
394		F	34.5
395		E	41.5
396		S	94.1
397		Y	31.0
398		N	83.1
399		V	52.4
400		L	73.0
401		I	65.7
402		P	62.8
403		F	79.4

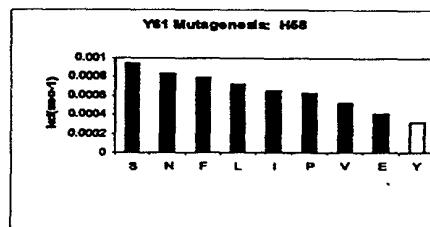
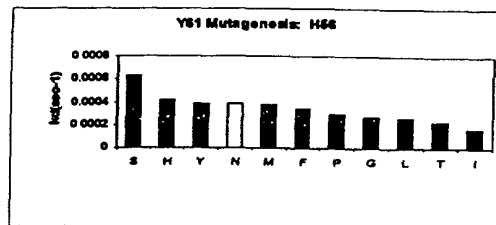


Figure 2D. Y61 Heavy Chain CDR H3 Mutagenesis

SEQ ID NO:		CDR H3						K_{off} ($\times 10^5$)
		96	96	97	98	101	102	
17	Y61	H	G	S	H	D	N	
404		E	231.5
405		S	193.0
406		H	28.7
407		K	227.5
408		Q	85.9
409		T	202.0
410		A	150.0
411		L	147.5
412		P	471.0
413		F	514.0
414		.	D	223.5
415		.	C	24.2
416		.	H	23.7
417		.	R	96.2
418		.	T	186.0
419		.	G	39.7
420		.	V	38.2
421		.	M	204.5
422		.	L	261.0
423		.	I	207.5
424		.	P	129.0
425		.	W	197.0
426		.	.	D	.	.	.	202.0
427		.	.	S	.	.	.	37.5
428		.	.	Y	.	.	.	273.0
429		.	.	H	.	.	.	190.5
430		.	.	R	.	.	.	224.0
431		.	.	N	.	.	.	221.5
432		.	.	T	.	.	.	58.8
433		.	.	G	.	.	.	229.0
434		.	.	A	.	.	.	143.0
435		.	.	I	.	.	.	208.0
436		.	.	P	.	.	.	300.0
437		.	.	W	.	.	.	239.0
438		.	.	F	.	.	.	180.5
439		.	.	.	H	.	.	25.5
440		.	.	.	R	.	.	34.0
441		.	.	.	T	.	.	22.7
442		.	.	.	A	.	.	67.3
443		.	.	.	V	.	.	29.3
444		.	.	.	L	.	.	59.8
445		.	.	.	I	.	.	34.3
446		.	.	.	F	.	.	68.8
447		D	.	14.4
448		S	.	44.9
449		Y	.	465.0
450		H	.	327.0
451		R	.	110.0

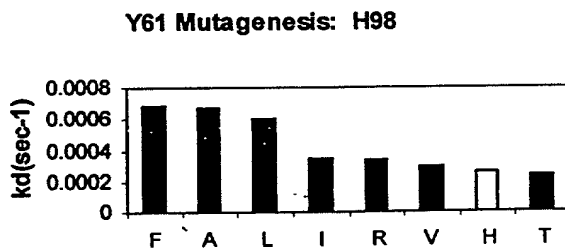
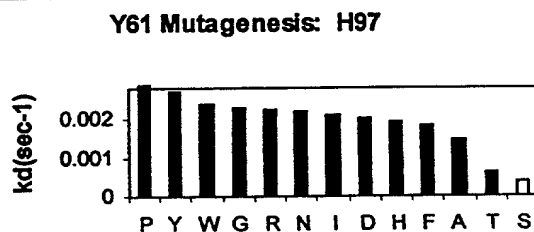
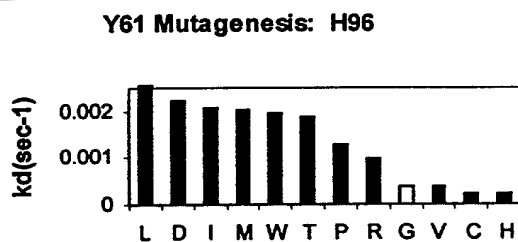
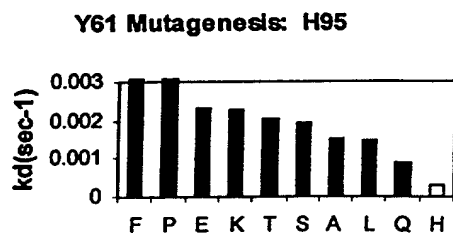


Figure 2E. Y61 Heavy Chain CDR H3 Mutagenesis

SEQ ID NO:		CDR H3						K_{off} ($\times 10^5$)
		95	96	97	98	101	102	
17	Y61	H	G	S	H	D	N	
452		N	.	223.0
453		G	.	375.0
454		A	.	106.5
455		V	.	163.0
456		I	.	162.5
457		S	32.5
458		H	18.0
459		K	40.5
460		R	57.5
461		N	40.3
462		T	33.3
463		G	69.2
464		A	38.2
465		L	95.6
466		I	99.6
467		P	181.5
468		W	23.5
469		F	31.8

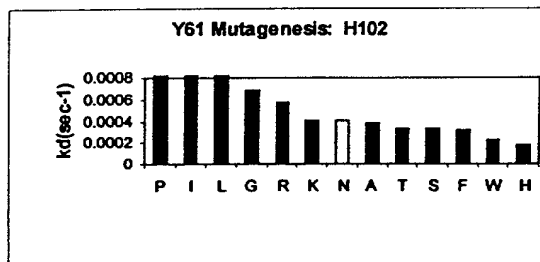
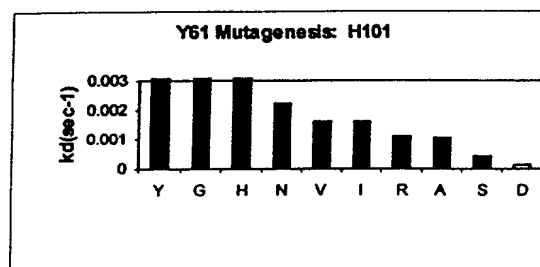


Figure 2F. Y61 Light Chain CDR L1 Mutagenesis

SEQ ID NO:		CDR L1													k_{off} ($\times 10^5$)
		24	25	26	27	27A	27B	28	29	30	31	32	33	34	
22	Y61	S	G	G	R	S	N	I	G	S	N	T	V	K	
470		D	22.0
471		C	18.6
472		S	21.1
473		Y	48.3
474		K	34.6
475		R	18.2
476		N	16.6
477		T	22.6
478		P	25.0
479		D	.	.	.	58.0
480		E	.	.	.	38.4
481		S	.	.	.	39.2
482		Y	.	.	.	35.7
483		H	.	.	.	31.5
484		K	.	.	.	33.1
485		N	.	.	.	22.9
486		Q	.	.	.	29.2
487		T	.	.	.	30.9
488		G	.	.	.	36.6
489		M	.	.	.	17.4
490		I	.	.	.	9.7
491		D	.	.	25.2
492		C	.	.	381.5
493		S	.	.	191.0
494		Y	.	.	21.3
495		H	.	.	26.0
496		K	.	.	31.8
497		R	.	.	690.0
498		N	.	.	196.5
499		Q	.	.	247.0
500		T	.	.	24.1
501		A	.	.	190.5
502		V	.	.	164.5
503		L	.	.	215.5
504		I	.	.	154.0
505		P	.	.	42.4

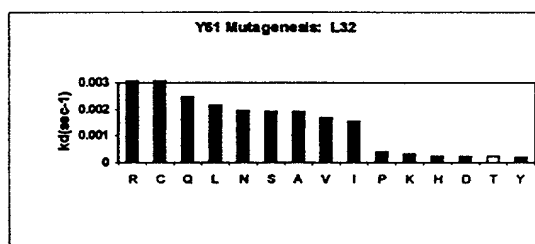
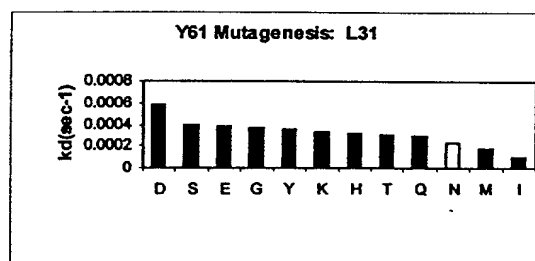
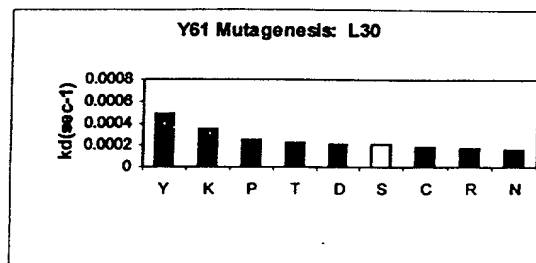


Figure 2G. Y61 Light Chain CDR L2 Mutagenesis

SEQ ID NO:		CDR L2							K_{off} ($\times 10^5$)
		S0	S1	S2	S3	S4	S5	S6	
20	Y61	G	N	D	Q	R	P	S	
506		D	34.8
507		E	61.7
508		C	46.7
509		S	28.6
510		Y	17.4
511		H	76.1
512		K	242.5
513		R	44.4
514		N	30.5
515		Q	34.8
516		T	27.2
517		G	21.5
518		A	37.2
519		V	38.5
520		M	95.3
521		L	61.6
522		I	120.5
523		P	41.0
524		W	38.2
525		F	3,476.7
526		.	.	.	S	.	.	.	86.6
527		.	.	.	Y	.	.	.	73.3
528		.	.	.	R	.	.	.	61.4
529		.	.	.	Q	.	.	.	29.7
530		.	.	.	T	.	.	.	83.4
531		.	.	.	A	.	.	.	55.4
532		.	.	.	I	.	.	.	85.5
533		.	.	.	P	.	.	.	97.4

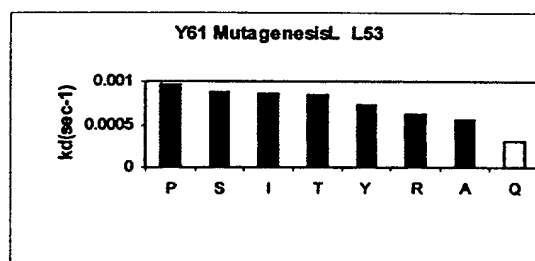
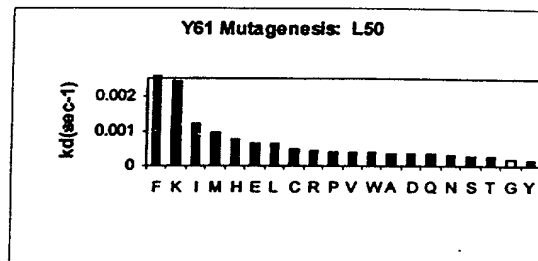


Figure 2H. Y61 Light Chain CDR L3 Mutagenesis

SEQ ID NO:	CDR L3												k_{off} ($\times 10^5$)
	89	90	91	92	93	94	95	95A	95B	95C	96	97	
18	Y61	Q	S	Y	D	R	G	T	H	P	A	L	L
534		.	.	.	D	25.9
535		.	.	.	C	45.3
536		.	.	.	S	30.7
537		.	.	.	Y	51.1
538		.	.	.	N	34.7
539		.	.	.	Q	42.7
540		.	.	.	T	40.8
541		.	.	.	G	34.9
542		.	.	.	A	35.7
543		.	.	.	L	72.8
544		.	.	.	I	61.8
545		.	.	.	W	72.0
546		.	.	.	F	44.9
547		.	.	.	D	34.3
548		.	.	.	C	32.0
549		.	.	.	S	34.1
550		.	.	.	Y	33.5
551		.	.	.	R	19.9
552		.	.	.	N	31.6
553		.	.	.	Q	30.0
554		.	.	.	T	31.6
555		.	.	.	G	39.2
556		.	.	.	A	31.0
557		.	.	.	V	26.9
558		.	.	.	M	27.5
559		.	.	.	L	30.0
560		.	.	.	I	29.5
561		.	.	.	P	34.9
562		.	.	.	W	34.9
563		D	25.3
564		C	52.0
565		S	28.7
566		Y	13.1
567		H	18.7
568		R	23.1
569		N	13.7
570		Q	25.0
571		T	30.5
572		G	25.6
573		A	52.6
574		V	35.1
575		L	24.4
576		I	27.6
577		P	33.2
578		W	29.3
579		F	23.6

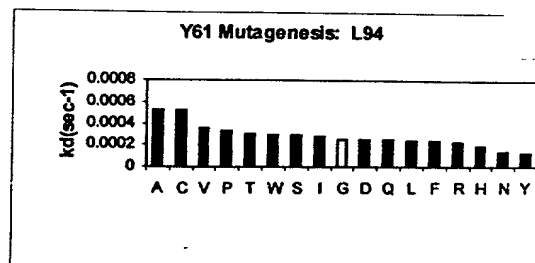
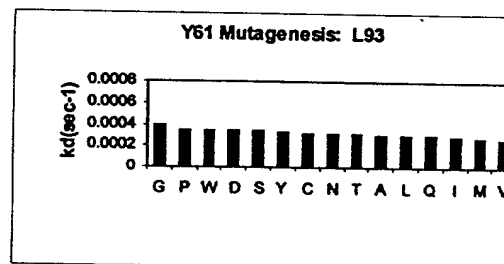
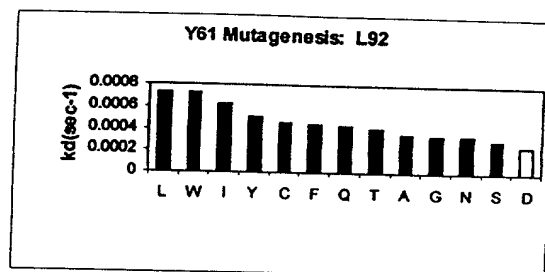
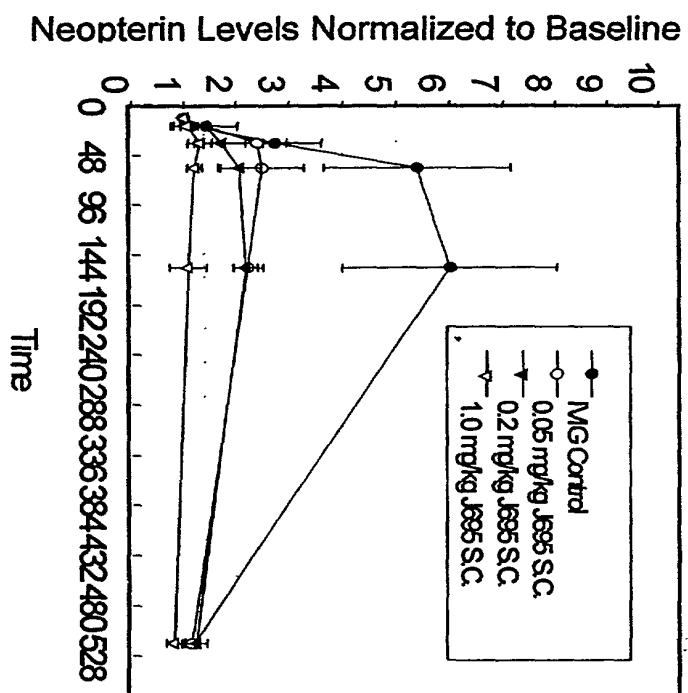
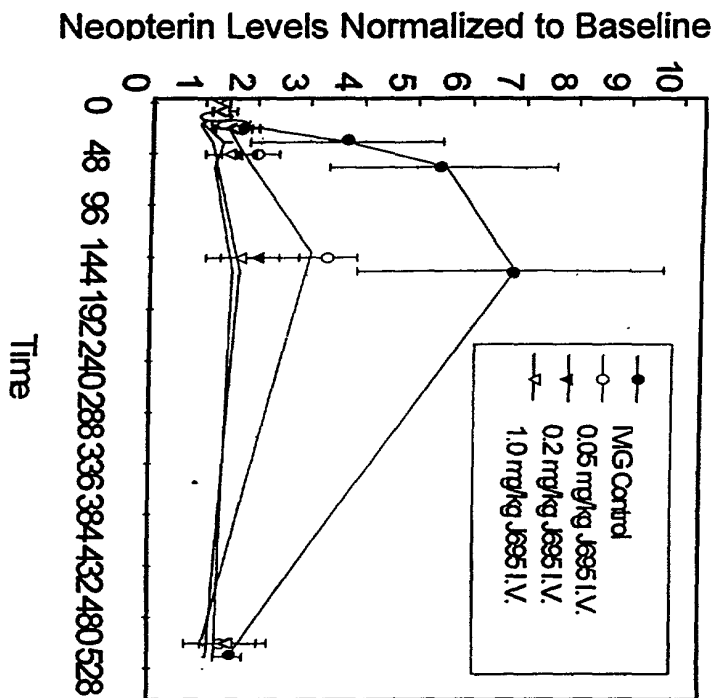


Figure 3: *In vivo* efficacy of J695 in cynomolgus monkeys



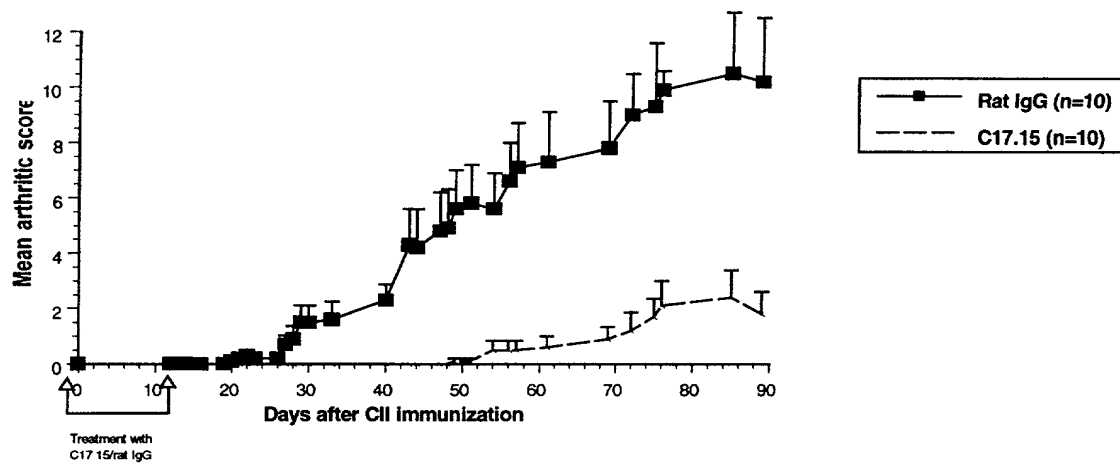


FIGURE 4

Declaration, Petition and Power of Attorney for Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**HUMAN ANTIBODIES THAT BIND HUMAN IL-12 AND
METHODS FOR PRODUCING**

the specification of which

(check one)

X is attached hereto.

 was filed on _____ as

Application Serial No. _____

and was amended on _____
(if applicable)

I do not know and do not believe that the subject matter of this application was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date of this application, or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date of this application on an application filed more than twelve months (six months if this application is for a design) before the filing of this application; and I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application, that no application for patent or inventor's certificate on the subject matter of this application has been filed by me or my representatives or assigns in any country foreign to the United States, except those identified below, and that I have reviewed and understand the contents of the specification, including the claims as amended by any amendment referred to herein.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

CLAIM OF BENEFIT OF EARLIER FOREIGN APPLICATION(S)

I hereby claim priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below, and have also identified below any foreign application(s) for patent or inventor's certificate filed by me on the same subject matter having a filing date before that of the application(s) from which priority is claimed.

Check one:

☒ no such applications have been filed.

☐ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
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			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

CLAIM FOR BENEFIT OF U.S. PROVISIONAL APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

60/126,603
(Application Serial No.)

March 25, 1999
(Filing Date)

(Application Serial No.)

(Filing Date)

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

I hereby claim the benefit under Title 35, United States Code, §120 of any earlier United States application(s) or PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the earlier application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date(s) of the earlier application(s) and the national or PCT international filing date of this application. As to subject matter of this application which is common to my earlier application(s), if any, described below, I do not know and do not believe that the same was known or used by others in the United States or patented or described in a printed publication in any country before my invention thereof, or patented or described in a printed publication in any country or in public use or on sale in the United States more than one year prior to the date(s) of said earlier application(s), or first patented or caused to be patented or made the subject of an inventor's certificate by me or my legal representatives or assigns in a country foreign to the United States prior to the date(s) of said earlier application(s) on an application filed more than twelve months (six months if this application is for a design) before the filing of said earlier application(s); and I acknowledge that no application for patent or inventor's certificate on said subject matter has been filed by me or my representatives or assigns in any country foreign to the United States except those identified herein.

<u>(Application Serial No.)</u>	<u>(Filing Date)</u>	<u>(Status)</u> (patented,pending,aband.)
<u>(Application Serial No.)</u>	<u>(Filing Date)</u>	<u>(Status)</u> (patented,pending,aband.)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Jane E. Remillard	Reg. No. 38,872	David J. Ridders	Reg. No. 43,882
Jeremiah Lynch	Reg. No. 17,425	Chi Suk Kim	Reg. No. 42,728
Kevin J. Canning	Reg. No. 35,470		
David A. Lane, Jr.	Reg. No. 39,261		

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Direct Telephone Calls to: Elizabeth A. Hanley, (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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SEQUENCE LISTING

<110> Jochen, Salfeld et al.

<120> Human Antibodies That Bind Human IL-12 And Methods For Producing

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<150> 60/126,603

<151> March 25, 1999

<160> 675

<170> PatentIn Ver. 2.0

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<211> 17
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Gly

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<223> Xaa at position 97 could be either Thr, Ala or Lys

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Xaa Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

'Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Asx
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Xaa Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Xaa Xaa Xaa Gly Ser Xaa Asp Xaa Trp Gly Gln Gly Thr Met Val Thr
100 105 110
Val Ser Ser
115

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<223> Xaa at position 54 could be either Gln or Asn

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35 40 45

Ile Tyr Xaa Asn Xaa Xaa Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Xaa Gln
65 70 75 80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Xaa Tyr Xaa Xaa Xaa Xaa
85 90 95

Xaa Xaa Xaa Xaa Xaa Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

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Gly

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          20                      25                      30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
      35                      40                      45
Ile Tyr Xaa Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
      50                      55                      60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Xaa Gln
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1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
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85 90 95
Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
100 105 110
Val Ser Ser
115

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20 25 30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Thr
85 90 95
His Pro Ala Leu Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

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 1 5 10

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 Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
 1 5 10 15

Gly

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Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
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 85 90 95
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 100 105 110
 Val Ser Ser
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 20 25 30
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 35 40 45
 Ile Tyr Tyr Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
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 85 90 95
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 100 105 110

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 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

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Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
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Val Ser Ser
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35 40 45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
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100 105 110

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35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

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Val Ser Ser
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Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
      35                      40                      45

Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
      50                      55                      60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
  65                      70                      75                      80

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Ser Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
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<213> Homo sapiens

<400> 37
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1                               10                      15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                      25                      30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                      40                      45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
      50                      55                      60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65                      70                      75                      80

```


Val Ser Ser
115

<210> 40
<211> 112
<212> PRT
<213> Homo sapiens

<400> 40
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
20 25 30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Phe
85 90 95
Thr Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

<210> 41
<211> 115
<212> PRT
<213> Homo sapiens

<400> 41
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Thr Ser Gly Ser Tyr Asp Tyr Trp Gly Gln Gly Thr Met Val Thr
100 105 110

Val Ser Ser
115

<210> 42
<211> 112

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<212> PRT
<213> Homo sapiens
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```

<400> 42
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1                    5                10              15

Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
          20                25              30

Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
          35                40              45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50                55              60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65                70              75              80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
          85                90              95

Trp Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100                105              110

```

```
<210> 43
<211> 115
<212> PRT
<213> Homo sapiens
```

```

<400> 43
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1                               5                               10                               15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                               25                               30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                               40                               45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
      50                               55                               60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65                               70                               75                               80
Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85                               90                               95
Thr Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
      100                               105                               110
Val Ser Ser
      115

```

```
<210> 44
<211> 112
<212> PRT
<213> Homo sapiens
```

<400> 44
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Phe
 85 90 95
 Thr Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 45
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 45
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Thr Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 46
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 46
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
 85 90 95
 Trp Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
 100 105 110

<210> 47
 <211> 115
 <212> PRT
 <213> Homo sapiens

<400> 47
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 48
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 48
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
 20 25 30
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
 65 70 75 80

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Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Thr Tyr Asp Lys Gly Phe
85 90 95
Thr Gly Ser Ser Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

<210> 49
<211> 115
<212> PRT
<213> Homo sapiens

<400> 49
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
100 105 110
Val Ser Ser
115

<210> 50
<211> 112
<212> PRT
<213> Homo sapiens

<400> 50
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
20 25 30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Thr Tyr Asp Lys Gly Phe
85 90 95
Thr Gly Ser Ser Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

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<210> 53

<211> 115
<212> PRT
<213> Homo sapiens

<400> 53
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
100 105 110
Val Ser Ser
115

<210> 54
<211> 112
<212> PRT
<213> Homo sapiens

<400> 54
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Val Ser Asn
20 25 30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Phe
85 90 95
Thr Gly Ser Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

<210> 55
<211> 115
<212> PRT
<213> Homo sapiens

<400> 55

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```

<400> 56
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
 1              5              10              15
Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Val Ser Asn
          20              25              30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
          35              40              45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50              55              60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65              70              75              80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Phe
          85              90              95
Thr Gly Ala Arg Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
          100              105              110

```

<400> 57
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
100 105 110
Val Ser Ser
115

<210> 58
<211> 112
<212> PRT
<213> Homo sapiens

<400> 58
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
20 25 30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Thr Tyr Asp Lys Gly Phe
85 90 95
Thr Gly Ser Ser Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

<210> 59
<211> 115
<212> PRT
<213> Homo sapiens

<400> 59
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
100 105 110
Val Ser Ser
115

<210> 60
<211> 112
<212> PRT
<213> Homo sapiens

<400> 60
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
20 25 30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Glu Arg Gly Phe
85 90 95
Thr Gly Ser Met Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

<210> 61
<211> 115
<212> PRT
<213> Homo sapiens

<400> 61
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

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<400> 64

Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
20 25 30

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65 70 75 80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Ser
85 90 95

His Pro Ala Leu Thr Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

<210> 65

<211> 115

<212> PRT

<213> Homo sapiens

<400> 65

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
100 105 110

Val Ser Ser
115

<210> 66

 $\langle 211 \rangle$ 112

<212> PRT

<213> Homo sapiens

<400> 66

Ser	Tyr	Val	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Gly	Thr	Pro	Gly	Gln
1				5					10					15	
Arg	Val	Thr	Ile	Ser	Cys	Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Ser	Asn
			20					25					30		
Thr	Val	Lys	Trp	Tyr	Gln	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu
		35					40					45			
Ile	Tyr	Gly	Asn	Asp	Gln	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser
	50					55					60				
Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala	Ser	Leu	Ala	Ile	Thr	Gly	Val	Gln
65					70					75					80
Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ser	Tyr	Asp	Arg	Gly	Thr
				85					90					95	
His	Pro	Leu	Thr	Met	Phe	Gly	Thr	Gly	Thr	Lys	Val	Thr	Val	Leu	Gly
			100					105					110		

<210> 67

<211> 115

<212> PRT

<213> Homo sapiens

<400> 67

[illegible]

<210> 68

$\langle 211 \rangle$ 112

<212> PRT

<213> Homo sapiens

<400> 68

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
20 25 30

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<210> 69
<211> 115
<212> PRT
<213> Homo sapiens
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```
<210> 70
<211> 112
<212> PRT
<213> Homo sapiens
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<400> 70
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
  1                               10                      15

Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
      20                      25                      30

Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
      35                      40                      45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
  50                      55                      60

```

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Thr
85 90 95
His Pro Ala Leu Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly
100 105 110

<210> 71
<211> 115
<212> PRT
<213> Homo sapiens

<400> 71
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Glu Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr
100 105 110
Val Ser Ser
115

<210> 72
<211> 112
<212> PRT
<213> Homo sapiens

<400> 72
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15
Arg Val Thr Ile Ser Cys Ser Gly Ser Arg Ser Asn Ile Gly Ser Asn
20 25 30
Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45
Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65 70 75 80
Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Gly Thr
85 90 95

<400> 74																
Gln	Ser	Val	Leu	Thr	Gln	Pro	Pro	Ser	Val	Ser	Gly	Ala	Pro	Gly	Gln	
1				5					10					15		
Arg	Val	Thr	Ile	Ser	Cys	Ser	Gly	Ser	Arg	Ser	Asn	Ile	Gly	Ser	Asn	
			20					25					30			
Thr	Val	Lys	Trp	Tyr	Gln	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu	
		35					40					45				
Ile	Tyr	Tyr	Asn	Asp	Gln	Arg	Pro	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	
	50					55					60					
Gly	Ser	Lys	Ser	Gly	Thr	Ser	Ala	Ser	Leu	Ala	Ile	Thr	Gly	Leu	Gln	
65					70					75					80	
Ala	Glu	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Gln	Ser	Tyr	Asp	Arg	Gly	Thr	
				85					90					95		
His	Pro	Ala	Leu	Leu	Phe	Gly	Thr	Gly	Thr	Lys	Val	Thr	Val	Leu	Gly	
			100					105					110			

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<210> 77
<211> 6
<212> PRT
<213> Homo sapiens
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[illegible]

$\langle 210 \rangle$	84
$\langle 211 \rangle$	12

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<400> 90
Thr Thr His Gly Ser His Asp Asn Trp Ser Gln Gly
  1                      5                      10
```


<210> 91
<211> 12
<212> PRT
<213> Homo sapiens

<400> 91
Thr Thr His Arg Ser His Asn Asn Trp Gly Gln Gly
1 5 10

<210> 92
<211> 8
<212> PRT
<213> Homo sapiens

<400> 92
Thr Thr His Gly Ser His Asp Asn
1 5

<210> 93
<211> 8
<212> PRT
<213> Homo sapiens

<400> 93
Thr Thr His Gly Ser His Asp Thr
1 5

<210> 94
<211> 8
<212> PRT
<213> Homo sapiens

<400> 94
Thr Lys His Gly Ser His Asp Asn
1 5

<210> 95
<211> 8
<212> PRT
<213> Homo sapiens

<400> 95
Thr Thr Gln Gly Arg His Asp Asn
1 5

<210> 96
<211> 8
<212> PRT
<213> Homo sapiens

<400> 96
Lys Thr Arg Gly Arg His Asp Asn
1 5

<210> 97
<211> 8
<212> PRT
<213> Homo sapiens

<400> 97

Thr Thr His Gly Ser His Asp Lys
1 5

<210> 98
<211> 8
<212> PRT
<213> Homo sapiens

<400> 98
Thr Thr His Gly Ser His Asp Asp
1 5

<210> 99
<211> 8
<212> PRT
<213> Homo sapiens

<400> 99
Lys Thr His Gly Ser His Asp Asn
1 5

<210> 100
<211> 8
<212> PRT
<213> Homo sapiens

<400> 100
Lys Thr His Gly Ser His Asp Asn
1 5

<210> 101
<211> 8
<212> PRT
<213> Homo sapiens

<400> 101
Thr Thr His Gly Ser His Asp Asn
1 5

<210> 102
<211> 8
<212> PRT
<213> Homo sapiens

<400> 102
Thr Thr Ser Gly Ser Tyr Asp Tyr
1 5

<210> 103
<211> 8
<212> PRT
<213> Homo sapiens

<400> 103
Thr Thr His Gly Ser His Asp Asn
1 5

<210> 104
<211> 8
<212> PRT
<213> Homo sapiens

$$\begin{aligned} \langle 210 \rangle & 111 \\ \langle 211 \rangle & 12 \end{aligned}$$

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<212> PRT
<213> Homo sapiens

<400> 111
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Arg Val
 1                      5                      10

<210> 112
<211> 12
<212> PRT
<213> Homo sapiens

<400> 112
Gln Ser Tyr Asp Ser Ser Leu Arg Gly Ser Arg Val
 1                      5                      10

<210> 113
<211> 12
<212> PRT
<213> Homo sapiens

<400> 113
Gln Ser Tyr Asp Ser Ser Leu Thr Gly Ser Arg Val
 1                      5                      10

<210> 114
<211> 12
<212> PRT
<213> Homo sapiens

<400> 114
Gln Ser Tyr Asp Ser Ser Leu Trp Gly Ser Arg Val
 1                      5                      10

<210> 115
<211> 12
<212> PRT
<213> Homo sapiens

<400> 115
Gln Thr Tyr Asp Ile Ser Glu Ser Gly Ser Arg Val
 1                      5                      10

<210> 116
<211> 12
<212> PRT
<213> Homo sapiens

<400> 116
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Arg Val
 1                      5                      10

<210> 117
<211> 12
<212> PRT
<213> Homo sapiens

<400> 117
Gln Thr Tyr Asp Arg Gly Phe Thr Gly Ser Arg Val
 1                      5                      10

```

<400> 124
Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Arg Val
1 5 10

```
<210> 126
<211> 12
<212> PRT
<213> Homo sapiens
```

```
<210> 127
<211> 12
<212> PRT
<213> Homo sapiens
```

```
<210> 128
<211> 12
<212> PRT
<213> Homo sapiens
```

```
<210> 129
<211> 12
<212> PRT
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<213> Homo sapiens

<400> 515
Gln Asn Asp Gln Arg Pro Ser
1 5

<210> 516
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<400> 516
Thr Asn Asp Gln Arg Pro Ser
1 5

<210> 517
<211> 7
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<400> 517
Gly Asn Asp Gln Arg Pro Ser
1 5

<210> 518
<211> 7
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<400> 518
Ala Asn Asp Gln Arg Pro Ser
1 5

<210> 519
<211> 7
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<213> Homo sapiens

<400> 519
Val Asn Asp Gln Arg Pro Ser
1 5

<210> 520
<211> 7
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<400> 520
Met Asn Asp Gln Arg Pro Ser
1 5

<210> 521
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<400> 521
Leu Asn Asp Gln Arg Pro Ser
1 5

<210> 522
<211> 7
<212> PRT
<213> Homo sapiens

<400> 522
Ile Asn Asp Gln Arg Pro Ser
1 5

<210> 523
<211> 7
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<400> 523
Pro Asn Asp Gln Arg Pro Ser
1 5

<210> 524
<211> 7
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<400> 524
Trp Asn Asp Gln Arg Pro Ser
1 5

<210> 525
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<212> PRT
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<400> 525

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<211> 7
<212> PRT
<213> Homo sapiens
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$\langle 210 \rangle$	539
$\langle 211 \rangle$	12

<213> Homo sapiens

Gln Ser Tyr Gln Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<211> 12

<213> Homo sapiens

Gln Ser Tyr Thr Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<211> 12

<213> Homo sapiens

Gln Ser Tyr Gly Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<211> 12

<213> Homo sapiens

Gln Ser Tyr Ala Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<211> 12

<213> Homo sapiens

Gln Ser Tyr Leu Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<211> 12

<213> Homo sapiens

Gln Ser Tyr Ile Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<211> 12

<213> Homo sapiens

Gln Ser Tyr Trp Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 546
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<213> Homo sapiens

<400> 546
Gln Ser Tyr Phe Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 547
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<212> PRT
<213> Homo sapiens

<400> 547
Gln Ser Tyr Asp Asp Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 548
<211> 12
<212> PRT
<213> Homo sapiens

<400> 548
Gln Ser Tyr Asp Cys Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 549
<211> 12
<212> PRT
<213> Homo sapiens

<400> 549
Gln Ser Tyr Asp Ser Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 550
<211> 12
<212> PRT
<213> Homo sapiens

<400> 550
Gln Ser Tyr Asp Tyr Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 551
<211> 12
<212> PRT
<213> Homo sapiens

<400> 551
Gln Ser Tyr Asp Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 552
<211> 12
<212> PRT
<213> Homo sapiens

<400> 552
Gln Ser Tyr Asp Asn Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 553
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 553
 Gln Ser Tyr Asp Gln Gly Thr His Pro Ala Leu Leu
 1 5 10

<210> 554
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 554
 Gln Ser Tyr Asp Thr Gly Thr His Pro Ala Leu Leu
 1 5 10

<210> 555
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 555
 Gln Ser Tyr Asp Gly Gly Thr His Pro Ala Leu Leu
 1 5 10

<210> 556
 <211> 12
 <212> PRT
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<400> 556
 Gln Ser Tyr Asp Ala Gly Thr His Pro Ala Leu Leu
 1 5 10

<210> 557
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 557
 Gln Ser Tyr Asp Val Gly Thr His Pro Ala Leu Leu
 1 5 10

<210> 558
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 558
 Gln Ser Tyr Asp Met Gly Thr His Pro Ala Leu Leu
 1 5 10

<210> 559
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 <212> PRT
 <213> Homo sapiens

<400> 559

Gln Ser Tyr Asp Leu Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 560
<211> 12
<212> PRT
<213> Homo sapiens

<400> 560
Gln Ser Tyr Asp Ile Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 561
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<212> PRT
<213> Homo sapiens

<400> 561
Gln Ser Tyr Asp Pro Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 562
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<213> Homo sapiens

<400> 562
Gln Ser Tyr Asp Trp Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 563
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<212> PRT
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<400> 563
Gln Ser Tyr Asp Arg Asp Thr His Pro Ala Leu Leu
1 5 10

<210> 564
<211> 12
<212> PRT
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<400> 564
Gln Ser Tyr Asp Arg Cys Thr His Pro Ala Leu Leu
1 5 10

<210> 565
<211> 12
<212> PRT
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<400> 565
Gln Ser Tyr Asp Arg Ser Thr His Pro Ala Leu Leu
1 5 10

<210> 566
<211> 12
<212> PRT
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<400> 566
Gln Ser Tyr Asp Arg Tyr Thr His Pro Ala Leu Leu
1 5 10

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<210> 567
<211> 12
<212> PRT
<213> Homo sapiens
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<400> 567
Gln Ser Tyr Asp Arg His Thr His Pro Ala Leu Leu
1 5 10

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<210> 568
<211> 12
<212> PRT
<213> Homo sapiens
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<400> 568
Gln Ser Tyr Asp Arg Arg Thr His Pro Ala Leu Leu
1 5 10

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<210> 569
<211> 12
<212> PRT
<213> Homo sapiens
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<400> 569
Gln Ser Tyr Asp Arg Asn Thr His Pro Ala Leu Leu
1 5 10

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<210> 570
<211> 12
<212> PRT
<213> Homo sapiens
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<400> 570
Gln Ser Tyr Asp Arg Gln Thr His Pro Ala Leu Leu
1 5 10

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<210> 571
<211> 12
<212> PRT
<213> Homo sapiens
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<400> 571
Gln Ser Tyr Asp Arg Thr Thr His Pro Ala Leu Leu
1 5 10

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<210> 572
<211> 12
<212> PRT
<213> Homo sapiens
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<400> 572
Gln Ser Tyr Asp Arg Gly Thr His Pro Ala Leu Leu
1 5 10

<210> 573
<211> 12

<400> 579
Gln Ser Tyr Asp Arg Phe Thr His Pro Ala Leu Leu
1 5 10

<210> 580
 <211> 48
 <212> DNA
 <213> synthetic construct
 <223> nucleotides at positions 16 to 34 can be substituted with any nucleotide such that the randomized nucleotides represent 12% of the sequence

 <400> 580
 tgtcccttgg cccagtagt catagctccc actggtcgta cagtaata 48

 <210> 581
 <211> 35
 <212> DNA
 <213> synthetic construct

 <400> 581
 gacacctcga tcagcggata acaatttcac acagg 35

 <210> 582
 <211> 15
 <212> DNA
 <213> synthetic construct

 <400> 582
 tggggccaag ggaca 15

 <210> 583
 <211> 45
 <212> DNA
 <213> synthetic construct

 <400> 583
 attcgtccta taccgttcta cttgtcgtc tttccagacg ttagt 45

 <210> 584
 <211> 18
 <212> DNA
 <213> synthetic construct

 <400> 584
 attcgtccta taccgttc 18

 <210> 585
 <211> 66
 <212> DNA
 <213> synthetic construct
 <223> nucleotides from position 28 to 42 can be substituted with any nucleotide such that the randomized nucleotides represent 12% of the sequence

 <400> 585
 ggtcccagtt ccgaagaccc tcgaaccct caggctgctg tcatatgact ggcagtaata 60
 gtcagc 66

 <210> 586
 <211> 15
 <212> DNA
 <213> synthetic construct

 <400> 586
 tggggccaag ggaca 15

 <210> 587
 <211> 24
 <212> DNA

<213> synthetic construct

<400> 587

tgaagagacg gtgaccattg tccc

24

<210> 588

<211> 16

<212> DNA

<213> synthetic construct

<400> 588

gacacctcga tcagcg

16

<210> 589

<211> 48

<212> DNA

<213> synthetic construct

<400> 589

gagtcattct cgacttgctg cgcacctag gacggtcagc ttggtccc

48

<210> 590

<211> 12

<212> PRT

<213> Homo sapiens

<400> 590

Gln Ser Tyr Asp Arg Gly Phe Thr Gly Ser Met Val
1 5 10

<210> 591

<211> 12

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa is encoded by a randomized codon of sequence
NNS with N being any nucleotide and S being either
deoxycytosine or deoxyguanine

<400> 591

Xaa Xaa Xaa Xaa Xaa Xaa Phe Thr Gly Ser Met Val
1 5 10

<210> 592

<211> 12

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa is encoded by a randomized codon of sequence
NNS with N being any nucleotide and S being either
deoxycytosine or deoxyguanine

<400> 592

Gln Ser Tyr Xaa Xaa Xaa Xaa Xaa Xaa Ser Met Val
1 5 10

<210> 593

<211> 12

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa is encoded by a randomized codon of sequence

NNS with N being any nucleotide and S being either deoxycytosine or deoxyguanine

<400> 593

Gln Ser Tyr Asp Arg Gly Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10

<210> 594

 $\langle 211 \rangle$ 100

<212> PRT

<213> Homo sapiens

<400> 594

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
20 25 30

Tyr Met Asp Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Gly Arg Thr Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg
100

<210> 595

 $\langle 211 \rangle$ 100

<212> PRT

<213> Homo sapiens

<400> 595

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
20 25 30

Tyr Met Ser Trp Val Arg Gln Ala Gln Gly Lys Gly Leu Glu Leu Val
35 40 45

Gly Leu Ile Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
50 55 60

Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr
65 70 75 80

Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr
85 90 95

Tyr Cys Ala Arg
100

<210> 596

<211> 100

<212> PRT

<213> Homo sapiens

Variable	Mean	SD	Min	Max	Skewness	Kurtosis	Normality
Age	35.2	12.5	18	65	0.15	3.2	0.98
Gender	1.2	0.4	1	2	0.05	3.0	0.99
Marital Status	1.5	0.5	1	3	0.10	3.1	0.98
Education	12.5	2.5	8	16	0.20	3.3	0.97
Income	1500	500	500	3000	0.30	3.5	0.96
Occupation	1.8	0.6	1	3	0.15	3.2	0.98
Health Status	1.2	0.4	1	2	0.05	3.0	0.99
Stress Level	2.5	1.0	1	4	0.25	3.4	0.97
Life Satisfaction	3.5	1.5	1	5	0.35	3.6	0.96
Resilience	2.8	1.2	1	4	0.20	3.3	0.97
Optimism	3.2	1.3	1	4	0.25	3.4	0.97
Emotional Stability	2.0	0.8	1	3	0.10	3.1	0.98
Self-Esteem	3.0	1.0	1	4	0.20	3.3	0.97
Life Purpose	2.5	1.0	1	4	0.25	3.4	0.97
Gratitude	3.0	1.2	1	4	0.20	3.3	0.97
Forgiveness	2.8	1.1	1	4	0.20	3.3	0.97
Empathy	3.2	1.3	1	4	0.25	3.4	0.97
Resilience	2.8	1.2	1	4	0.20	3.3	0.97
Optimism	3.2	1.3	1	4	0.25	3.4	0.97
Emotional Stability	2.0	0.8	1	3	0.10	3.1	0.98
Self-Esteem	3.0	1.0	1	4	0.20	3.3	0.97
Life Purpose	2.5	1.0	1	4	0.25	3.4	0.97
Gratitude	3.0	1.2	1	4	0.20	3.3	0.97
Forgiveness	2.8	1.1	1	4	0.20	3.3	0.97
Empathy	3.2	1.3	1	4	0.25	3.4	0.97

<400> 596

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
20 25 30

Tyr Met Ser Trp Val Arg Gln Ala Gln Gly Lys Gly Leu Glu Leu Val
35 40 45

Gly Leu Ile Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
50 55 60

Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr
65 70 75 80

Met Tyr Leu Gln Met Ser Asn Leu Lys Thr Glu Asp Leu Ala Val Tyr
85 90 95

Tyr Cys Ala Arg
100

<210> 597

<211> 100

<212> PRT

<213> Homo sapiens

<400> 597

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp His
20 25 30

Tyr Met Ser Trp Val Arg Gln Ala Gln Gly Lys Gly Leu Glu Leu Val
35 40 45

Gly Leu Ile Arg Asn Lys Ala Asn Ser Tyr Thr Thr Glu Tyr Ala Ala
50 55 60

Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr
65 70 75 80

Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr
85 90 95

Tyr Cys Ala Arg
100

<210> 598

<211> 98

<212> PRT

<213> Homo sapiens

<400> 598

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

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Ser Gly Ile Ser Trp Asn Ser Gly Ser Ile Gly Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

Ala Lys

<210> 599
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 599
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30
 Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr His Cys
 85 90 95

Ala Arg

<210> 600
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 600
 Glu Val Gln Leu Val Glu Ser Gly Gly Val Val Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
 20 25 30
 Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Leu Ile Ser Trp Asp Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Thr Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

Ala Lys

<210> 601
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 601
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30
 Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 602
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 602
 Gln Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
 20 25 30
 Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Ser Ser Ser Ser Tyr Thr Asn Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 603
 <211> 100
 <212> PRT
 <213> Homo sapiens

<400> 603
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gly Ser
 20 25 30

Ala Met His Trp Val Arg Gln Ala Ser Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Arg Ile Arg Ser Lys Ala Asn Ser Tyr Ala Thr Ala Tyr Ala Ala
50 55 60
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Ala Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Thr Arg
100

<210> 604
<211> 100
<212> PRT
<213> Homo sapiens

<400> 604
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
50 55 60
Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95
Tyr Cys Thr Thr
100

<210> 605
<211> 100
<212> PRT
<213> Homo sapiens

<400> 605
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
20 25 30
Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Gly Arg Ile Glu Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
50 55 60
Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
65 70 75 80
Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
85 90 95

<400> 608

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
 50 55 60
 Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
 65 70 75 80
 Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95
 Tyr Cys Thr Thr
 100

<210> 609
 <211> 100
 <212> PRT
 <213> Homo sapiens

<400> 609
 Glu Val Gln Leu Val Glu Ser Gly Gly Ala Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ala
 20 25 30
 Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Gly Arg Ile Lys Ser Lys Thr Asp Gly Gly Thr Thr Asp Tyr Ala Ala
 50 55 60
 Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr
 65 70 75 80
 Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr
 85 90 95
 Tyr Cys Thr Thr
 100

<210> 610
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 610
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Pro Ala Ser Gly Phe Thr Phe Ser Asn His
 20 25 30
 Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Tyr Ile Ser Gly Asp Ser Gly Tyr Thr Asn Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Asn Asn Ser Pro Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Lys

<210> 611
<211> 98
<212> PRT
<213> Homo sapiens

<400> 611
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn His
20 25 30

Tyr Thr Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ser Ser Gly Asn Ser Gly Tyr Thr Asn Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Lys

<210> 612
<211> 98
<212> PRT
<213> Homo sapiens

<400> 612
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ser
20 25 30

Asp Met Asn Trp Val His Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Ile Ile Ser Arg Asp Asn Ser Arg Asn Thr Leu Tyr
65 70 75 80

Leu Gln Thr Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Arg

<210> 613
<211> 98

<212> PRT
 <213> Homo sapiens

<400> 613

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ser
 20 25 30
 Asp Met Asn Trp Ala Arg Lys Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Val Asp Ser Val
 50 55 60
 Lys Arg Arg Phe Ile Ile Ser Arg Asp Asn Ser Arg Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Lys Asn Arg Arg Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys
 85 90 95
 Val Arg

<210> 614
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 614

Thr Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Glu Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Ser
 20 25 30
 Asp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Val Ser Trp Asn Gly Ser Arg Thr His Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Ile Ile Ser Arg Asp Asn Ser Arg Asn Phe Leu Tyr
 65 70 75 80
 Gln Gln Met Asn Ser Leu Arg Pro Glu Asp Met Ala Val Tyr Tyr Cys
 85 90 95
 Val Arg

<210> 615
 <211> 97
 <212> PRT
 <213> Homo sapiens

<400> 615

Glu Val His Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ala Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30
 Asp Met His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Asn Gly Thr Ala Gly Asp Thr Tyr Tyr Pro Gly Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

<210> 616

<211> 97

<212> PRT

<213> Homo sapiens

<400> 616

Glu Val Gln Leu Val Glu Thr Gly Gly Gly Leu Ile Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Val Ser Ser Asn
20 25 30

Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Val Ile Tyr Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

<210> 617

<211> 97

<212> PRT

<213> Homo sapiens

<400> 617

Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val His Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Gly Thr Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

<210> 618
 <211> 97
 <212> PRT
 <213> Homo sapiens

<400> 618
 Glu Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Ile Gly Thr Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
 65 70 75 80
 Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys Ala
 85 90 95

Arg

<210> 619
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 619
 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys

<210> 620
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 620
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Val Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Val Lys

<210> 621
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 621
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Val Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Val Lys

<210> 622
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 622
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asn Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

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<210> 625
<211> 98
<212> PRT
<213> Homo sapiens
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[illegible]

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<211> 98

<212> PRT

<213> Homo sapiens

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<211> 98

<212> PRT

<213> Homo sapiens

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 628

<211> 98

<212> PRT

<213> Homo sapiens

<400> 628

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 629

<211> 98

<212> PRT

<213> Homo sapiens

<400> 629

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 630

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<400> 630
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1              5              10              15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20              25              30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35              40              45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
      50              55              60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65              70              75              80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
          85              90              95

Ala Arg

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<210> 631
<211> 98
<212> PRT
<213> Homo sapiens

<400> 631
Gln Val Gln Leu Val Glu Ser Gly Gly Val Val Gln Pro Gly Arg
  1             5             10             15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20             25             30
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35             40             45
Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
          50             55             60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
  65             70             75             80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
          85             90             95
Ala Arg

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<210> 632
<211> 98
<212> PRT
<213> Homo sapiens
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<400> 632
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 633
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 633
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Val Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Val Lys

<210> 634
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 634
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
 35 40 45
 Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 635
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 635
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Ala Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 636
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 636
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 637
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 637
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys

<210> 638
 <211> 97
 <212> PRT
 <213> Homo sapiens

<400> 638
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Asp Met His Trp Val Arg Gln Ala Thr Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ala Ile Gly Thr Ala Gly Asp Thr Tyr Tyr Pro Gly Ser Val Lys
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn Ser Leu Tyr Leu
 65 70 75 80

Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95

Arg

<210> 639
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 639
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Glu Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 640

<211> 98

<212> PRT

<213> Homo sapiens

<400> 640

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Leu Arg Ala Arg Leu Cys Ile Thr Val
85 90 95

Arg Glu

<210> 641

<211> 98

<212> PRT

<213> Homo sapiens

<400> 641

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 642

<211> 98

<212> PRT

<213> Homo sapiens

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<400> 642

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 643

<211> 98

<212> PRT

<213> Homo sapiens

<400> 643

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 644

<211> 98

<212> PRT

<213> Homo sapiens

<400> 644

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

<210> 647
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 647
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 648
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 648
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Gly Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 649
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 649
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

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<210> 650
<211> 98
<212> PRT
<213> Homo sapiens
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<400> 650
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1                               5                               10                               15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                               25                               30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                               40                               45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
      50                               55                               60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65                               70                               75                               80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85                               90                               95

Ala Lys

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<210> 651
<211> 98
<212> PRT
<213> Homo sapiens
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<400> 651
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
  1                      5                      10                      15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                      25                      30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
      35                      40                      45
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
      50                      55                      60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
      65                      70                      75                      80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85                      90                      95

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Ala Arg

<210> 652
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 652
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Lys

<210> 653
 <211> 95
 <212> PRT
 <213> Homo sapiens

<400> 653
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Arg Lys
 85 90 95

<210> 654
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 654
 Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg

<210> 655
<211> 98
<212> PRT
<213> Homo sapiens

<400> 655
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Ala
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Thr Asn Thr Leu Phe
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Arg

<210> 656
<211> 98
<212> PRT
<213> Homo sapiens

<400> 656
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 657
<211> 98
<212> PRT
<213> Homo sapiens

<400> 657
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 658
<211> 97
<212> PRT
<213> Homo sapiens

<400> 658
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ser Ile Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

<210> 659
<211> 98
<212> PRT
<213> Homo sapiens

<400> 659

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Ser Ile Ser Ser Ser Ser Ser Tyr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 660

<211> 98

<212> PRT

<213> Homo sapiens

<400> 660

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ser Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Tyr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 661

<211> 97

<212> PRT

<213> Homo sapiens

<400> 661

Glu Asp Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Pro Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ser Tyr
 20 25 30

Val Leu His Trp Val Arg Arg Ala Pro Gly Lys Gly Pro Glu Trp Val
 35 40 45

Ser Ala Ile Gly Thr Gly Gly Asp Thr Tyr Tyr Ala Asp Ser Val Met
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Lys Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Ile Ala Glu Asp Met Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

<210> 662

<211> 98

<212> PRT

<213> Homo sapiens

<400> 662

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
35 40 45

Ser Arg Ile Asn Ser Asp Gly Ser Ser Thr Ser Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 663

<211> 98

<212> PRT

<213> Homo sapiens

<400> 663

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
35 40 45

Ser Arg Ile Asn Ser Asp Gly Ser Ser Thr Ser Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 664

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<400> 664
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
  1                      5                      10                      15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20                      25                      30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
    35                      40                      45

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
    50                      55                      60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
    65                      70                      75                      80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
      85                      90                      95

Ala Arg

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<210> 665
<211> 98
<212> PRT
<213> Homo sapiens

<400> 665
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1             5             10             15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20             25             30
Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
      35             40             45
Ser Arg Ile Asn Ser Asp Gly Ser Ser Thr Ser Tyr Ala Asp Ser Met
      50             55             60
Lys Gly Gln Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
      65             70             75             80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys
      85             90             95
Thr Arg

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<210> 666
<211> 98
<212> PRT
<213> Homo sapiens

<400> 666
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1             5             10             15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20             25             30

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<400> 668
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
  1                      5                10              15

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
      20                25              30

Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
      35                40              45

Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
      50                55              60

Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
      65                70              75              80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
      85                90              95

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<400> 671
Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
 20 25 30
 Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65 70 75 80
 Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95
 Ser Gly

<210> 672
 <211> 98
 <212> PRT
 <213> Homo sapiens

<400> 672
 Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Glu Ala Pro Arg Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
 20 25 30
 Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Lys Ala Pro Lys Leu Leu
 35 40 45
 Ile Tyr Tyr Asp Asp Leu Leu Pro Ser Gly Val Ser Asp Arg Phe Ser
 50 55 60
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Gln
 65 70 75 80
 Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu
 85 90 95
 Asn Gly

<210> 673
 <211> 99
 <212> PRT
 <213> Homo sapiens

<400> 673
 Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
 1 5 10 15
 Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
 20 25 30
 Tyr Val Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
 35 40 45
 Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Gln Phe
 50 55 60
 Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
 65 70 75 80

Leu Asn Ala

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<210> 674
<211> 99
<212> PRT
<213> Homo sapiens
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<400> 674
Gln Ser Val Val Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Ser Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser
85 90 95

Leu Ser Gly

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<210> 675
<211> 98
<212> PRT
<213> Homo sapiens
```

<400> 675
Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn
20 25 30

Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Gly Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Val Gln
65 70 75 80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
85 90 95

Arg Gly